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**Cover image:** Scorpion, *Mesobuthus martensii* (Karsch, 1879). Photo by Zhi-Yong DI

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## Stepping Stones: A New Future for *Zoological Research*

This past year has been an auspicious one for Chinese science. With the recent lunar landing and the advances and the Chinese Academy of Sciences' growing reputation as the leader of scientific publishing in Asia, the media is now constantly reporting the “great leaps” that Chinese researchers have made. But we would do well to remember an old Chinese proverb: “It is better to take many small steps in the right direction than to make a great leap forward only to stumble backward.” None of the recent advances of this year are great leaps in the sense that they occurred from nothing—rather, they are the fruits of many decades of hard work undertaken by our colleagues both in China and abroad. Every major advance is only a “leap” when one does not consider the many small steps taken to get there. In that spirit, we will be taking a leap of our own in 2014.

When we first began publishing *Zoological Research* (ZR) in 1980, nearly every major piece of scientific research was done in either the United States or Europe, with less than 1% of global research coming from China. This dearth of domestic innovation is what initially spurred us to begin publishing ZR as a Chinese-language journal that supported regional and national research initiatives aimed at developing the native Chinese scientific community. Over the next two decades, we diligently worked to make ZR one of the top journals in Western China, but by the mid 1990s, we began to see just what impact Chinese contributions were starting to have on the international scientific community as well as the benefits of foreign research being published domestically. This realization prompted us to open up submissions to include articles published in English by both Chinese and foreign scientists, and in 1996, we published our first set of English articles.

Since *Zoological Research* became a joint English-Chinese publication, we seen enormous growth, publishing work from researchers in over 60 different countries and becoming indexed by PubMed/Medline in addition to Biological Abstracts, Zoological Record, and Chemical Abstracts, among others. Our decision to pursue a dual-language journal was born out of a desire to continue building up the domestic research base for Chinese authors and serve as a bridge between our colleagues at home and the international community abroad. In examining both of those goals, the dual-language nature of the journal has succeeded immensely. But the situation today is very different than it was nearly twenty years ago. By sheer numbers, China is now the no. 2 global producer of research, accounting for 14% of all research in 2010 and publishing some 200,000+ articles annually. In terms of quality, China is projected to overtake both the UK and Germany by 2015. ZR itself has seen similar growth—this past year, our journal was awarded the highest honor a Chinese research publication can receive, “The Highest International Impact Academic Journals of China” for its achievement in internationalizing Chinese life science research, meaning that ZR was in the top 5% of some 3500+ science and technology periodicals published in mainland China, based on evaluating total international citation frequency and international impact factor.

While bringing *Zoological Research* to the fore of Chinese scientific publishing is impressive achievement, it is not a great leap forward, but just another of many steps towards making ZR a truly international platform for scientific research. In fact, I believe this merely a beginning in a much greater move forward. As China increasingly becomes a center for scientific innovation, we must embrace our position as a global leader in scientific research and work harder to create an internationally renowned innovation environment that will allow researchers from across the world to participate and contribute to our efforts. In the spirit of international cooperation that has deeply enriched the

scholarship of research published in our journal and in the belief that we can do better, we are pleased to announce that starting this year, *Zoological Research* will become an internationally aimed English-language only journal. While we are immensely proud of the work we have published in Chinese over the past 170 issues, we fully believe that there is no better time than now to take advantages of our journals two great strengths: a strong regional focus of the finest Chinese scholarship, and a growing internationally focused English-language research published by authors around the globe. We hope this new format both maintains our distinction as one of China's leading life science journals and embraces a new international outlook, looking toward the future but retaining the characteristics of our past. To aid in this endeavor, our sponsoring institution, the Kunming Institute of Zoology, has hired an in-house editor from the United States, who, over the coming year, will be offering complimentary English language editing on incoming research and working closely with submitting authors on refining their manuscripts to the caliber expected of an internationally recognized publication.

As part of our new English-language journal, we are especially interested in papers focusing on our key areas related to animals: Genome Evolution and Genetic Diversity, DNA Barcoding, Developmental Biology, Physiology, Biochemistry, Immunology, Neuroscience, Animal Ecology and Ethology, and especially Animal Models of Human Diseases. We would also like to make a special invitation to our readers to serve as single-issue Guest Editors for upcoming issues. Researchers interested in organizing special themed issues to explore a particular topic are heartily invited to contact our editorial office for more details. Likewise, to date, nearly a third of our editorial board is based outside of China. In moving *Zoological Research* forward as an international journal, we are especially interested in recruiting scientists who can bring their expertise to bear on our plans for the journal over the coming years. If you are interested in becoming an editor for *ZR*, please feel free to contact me personally at any time.

As always, thank you for your constant support of our journal and our work. Please continue sending your prospective manuscripts and following our journal as it enters an exciting new era. Let us take this next step together and make *Zoological Research* a respected forum for biological research in China and around the world.

Sincerely,



Yong-Gang YAO, Editor-in-Chief

(Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming 650223, China)

# History of study, updated checklist, distribution and key of scorpions (Arachnida: Scorpiones) from China

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**Abstract:** This review describes the history of taxonomic research on scorpions and provides an updated checklist and key of the scorpions currently known in China. This checklist is based on a thorough review of the extant literatures on scorpion species whose presence has been confirmed in China through field expeditions and examination of scorpion collections, excepting a few members that have no clear distribution or are currently in doubt. Totally, the scorpion fauna of China consists of 53 species and subspecies belonging to 12 genera crossing five families, with 33 species (62.3%) and one genus being recorded as endemic. Additionally, identification key and the distribution of scorpions from China are provided.

**Keywords:** Scorpion; Taxonomy; Checklist; Key; Distribution; China

China is comprised of a vast territory crossing frigid, temperate, and tropical zones, as well as complex topography (80% of which are mountains) including rich rivers, lakes, and diverse climate. These features contribute to China's possession of high species and habitat diversity, making it to be one of 12 megadiversity countries. For example, there are at the least  $1.5 \times 10^4$  species estimated conservatively, which is about 1/10 of the sum of the entire world (88 328, 929 050 species recorded in China and the world respectively, Yu, 2004).

Of these species, Scorpions, though as a small arachnid group, are quite interesting. To date, there are 15 families, 197 genera and 2 069 species recorded in the world (6/20/2013, <http://www.ntnu.no/ub/scorpion-files/index.php>). Aristotle (384–322 BC) was the first scorpion researcher who studied zoological information about biogeography of scorpions in the western world (Fet et al, 2009), a search enhanced by the systematics research began by Carl von Linne (1758), who recorded five scorpion species in the tenth edition of *Systema Naturae*, and classified them as the members of the genus *Scorpio* in Insect Aptera (Fet et al, 2002).

Chinese reported scorpions from 2 000 years ago, as

the simple information in *Er ya* (published in 221 BC–9 AD) (Wang & Chen, 2007). Scorpions are found in some multifarious ancient books and local chronicles, especially the traditional Chinese medicine books, such as, *Shu ben cao*, *Ben cao tu jing*, *Ben cao jing shu*, and *Ben cao bei yao* (Zhang et al, 2009). However, all of these historical data contained no value on classification. The beginning of Chinese scorpion taxonomy research was started by foreign scientists. Prior to 2003, there were 19 species and subspecies reported in China, as noted by Zhu et al (2004). *Buthus confucius* Karsch (1879) (= *Buthus martensii* = *Mesobuthus martensii*, belonging to the family Buthidae) was the first species described from China (Zhu et al, 2004). Simon (1880) described a new species, *Buthus confucius* (Buthidae), on the basis of specimens collected in Beijing (cited in Qi et

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al, 2004; Shi & Zhang, 2005). Kraepelin (1899) reviewed the order Scorpiones, including 13 species and subspecies from China. Karsch (1881) also suggested that *Buthus confucius* might be a synonym of *Buthus martensii* (Qi et al, 2004; Shi & Zhang, 2005). Birula (1897, 1911) named three subspecies from China: *Buthus caucasicus intermedius* (Birula, 1897) (= *Mesobuthus caucasicus intermedius*); *Buthus caucasicus przewalskii* (Birula, 1897) (= *Meso-buthus caucasicus przewalskii*); and *Buthus eupeus mongolcus* (Birula, 1911) (= *Mesobuthus eupeus mongolcus*). Birula's descriptions were backward and without normal figures. Birula (1904) described a new subspecies, *Buthus confucius hainanensis* (= *Mesobuthus martensii hainanensis*), based on a single specimen from Hainan Island. Wu (1936) identified two families, four genera and four species of scorpions from China. Kishida (1939) recorded Chinese scorpions and re-described *Buthus martensii*, however, his figures were very inaccurate, and he provided no characteristics of trichobothria. Takashima (1942, 1948, 1951) listed scorpions from Hainan and Shanxi, while Stahnke (1967) redescribed *Mesobuthus eupeus mongolcus*, but did not compare this species with its close relatives. Song et al (1982) finished his research on external morphology, reproductive system, life habits and development of *Buthus martensii*. Kovařík (1994) named a new subspecies of *Scorpiops hardwickii* (Gervais, 1843): *Scorpiops hardwickii jendeki* Kovařík, 1994, and later elevated this subspecies to species in his revision of the family Scorpiopidae ((transferred to Euscorpiidae by Sologlad & Sissom (2001)).

Though an impressive compilation of earlier scholarship, there were many limitations to these earlier studies. Thankfully, the number of research papers on Chinese scorpions increased gradually since the early 21st Century. Zhu et al (2004) published a list of Chinese scorpions based on Fet et al (2000) and Kishida (1939) mainly, listing five families, nine genera, 19 species and subspecies. Qi et al (2004) re-described *Mesobuthus martensii martensii* (Karsch, 1879) and provided the detailed study history of this species. Qi et al (2005) published the first comprehensive report on scorpions from Tibet, the authors discovered eight new species belonging to the family Chaerilidae (with one monotypic genus *Chaerilus*) and the family Euscorpiidae (the genera *Euscorpiops* and *Scorpiops*): *Chaerilus tessellatus* Qi, Zhu & Lourenço, 2005; *Euscorpiops karschi* Qi, Zhu & Lourenço, 2005; *Euscorpiops shidian*

Qi, Zhu & Lourenço, 2005; *Euscorpiops vachoni* Qi, Zhu & Lourenço, 2005; *Scorpiops atomatus* Qi, Zhu & Lourenço, 2005; *Scorpiops langxian* Qi, Zhu & Lourenço, 2005; *Scorpiops luridus* Qi, Zhu & Lourenço, 2005; and *Scorpiops pococki* Qi, Zhu & Lourenço, 2005. *Scorpiops petersii* Pocock, 1893 also appeared in this paper which followed on the report of Kishida (1939). Lourenço et al (2005) identified two new species: *Mesobuthus songi* Lourenço, Qi & Zhu, 2005 (= *Hottentotta songi* (Lourenço et al, 2005)), and *Heterometrus tibetanus* Lourenço, Qi, & Zhu, 2005 (belonging to the family Scorpionidae). Shi & Zhang (2005) summarized the research history of taxonomy of the family Buthidae and listed eight species and subspecies, four genera in China: *Mesobuthus martensii* (Karsch, 1879); *Mesobuthus eupeus* (Koch, 1839) (with two subspecies: *Mesobuthus eupeus mongolicus* (Birula, 1911); *Mesobuthus eupeus thersites* (C. L. Koch, 1839)); *Mesobuthus caucasicus* (Nordmann, 1840) (with two subspecies: *Mesobuthus caucasicus intermedius* (Birula, 1897); and *Mesobuthus caucasicus przewalskii* (Birula, 1897)); *Lychas mucronatus* (Fabricius, 1798); *Isometrus maculatus* (DeGeer, 1778); and *Orthochirus scrobiculosus* (Grube, 1873). Lourenço & Qi (2006) described one new genus and new species based on specimens from Tibet: *Tibetiomachus* Lourenço & Qi, 2006 (belonging to the family Hemiscorpidae), *Tibetiomachus himalayensis* Lourenço & Qi, 2006. Bastawade (2006) reported two new species and four new records based on specimens from Zangnan (the South Tibet, China): *Chaerilus dibangvalleycus* Bastawade, 2006; *Chaerilus pictus* (Pocock, 1890); *Chaerilus tricostatus* Pocock, 1899; *Euscorpiops asthenurus* (Pocock, 1900); *Euscorpiops kamengensis* Bastawade, 2006; and *Scorpiops leptochirus* Pocock, 1893. Zhu et al (2007) described a new species of *Euscorpiops* from Yunnan: *Euscorpiops yangi* Zhu, Zhang & Lourenço, 2007. Shi et al (2007) reported the geographical distribution of two species of *Mesobuthus*: *Mesobuthus eupeus* and *Mesobuthus martensii*. Zhu et al (2008) summarized the chaerilid scorpions of China, and provided the re-descriptions for *Chaerilus tessellatus* Qi, Zhu & Lourenço, 2005 and *Chaerilus triznai* Kovařík, 2000. They also pointed out that *Chaerilus pictus* (Pocock, 1890), which was described by Qi, Zhu & Lourenço (2005) was misidentified and described it as one new species: *Chaerilus conchiformis* Zhu, Han & Lourenço, 2008. Lourenço & Zhu (2008) discovered a new species

belonging to *Isometrus* (belonging to the family Buthidae): *Isometrus (Reddyanus) tibetanus* Zhu & Lourenço, 2008. Di & Zhu (2009a, b) described two new species: *Scorpiops lhasa* Di & Zhu, 2009; and *Chaerilus mainlingensis* Di & Zhu, 2009. Zhang & Zhu (2009) analysed the morphological variation of *Mesobuthus martensii* (Karsch, 1879) from Northern China and found that although this species is widespread in northern China, its morphology does not vary significantly and the variation both in males and females is below species level. Di & Zhu (2009c) described the male of *Euscorpiops karschi* for the first time. Di et al (2009) analysed the genus *Chaerilus* Simon, 1877 of China, with a description of the female *Chaerilus tricostatus* Pocock, 1899 for the first time (*Chaerilus assamensis* Kraepelin, 1913 was an erroneous record in this paper). Di & Zhu (2010) provided a redescription of *Scorpiops margerisonae* Kovařík, 2000 and described the female for the first time. Teruel & Rein (2010) transferred *Mesobuthus songi* Zhu, Qi & Lourenço, 2005 to the genus *Hottentotta* (belonging to the family Buthidae): *Hottentotta songi* (Zhu, Qi & Lourenço, 2005). Sun et al (2010) reported a new species of the genus *Mesobuthus* from Xinjiang, China: *Mesobuthus bolensis* Sun, Zhu & Lourenço, 2010, redescribed the characters of *Mesobuthus songi* Lourenço, Qi & Zhu, 2005, and transferred it to genus *Hottentotta* too. Di et al (2010a, b) discovered two new species in Yunnan: *Euscorpiops validus* Di, Cao, Wu & Li, 2010; *Euscorpiops puerensis* Di, Wu, Cao, Xiao & Li, 2010. Sun & Zhu (2010a, b) described new species of the

genera *Euscorpiops* and *Mesobuthus* from Yunnan and Xinjiang, China: *Euscorpiops xui* Sun & Zhu, 2010, and *Mesobuthus longichelus* Sun & Zhu, 2010. Lourenço et al (2010) published a new record genus and a new species in China: *Razianus xinjianganus* Lourenço, Sun & Zhu, 2010. Di et al (2011a) reviewed the scorpions from Yunnan, and described a new record species to China: *Euscorpiops kubani* Kovařík, 2004. Di et al (2011b) recorded the *Scorpiops* species distributing in Central China, and provided a key for the Chinese species of the genus. Sun & Sun (2011) reviewed the genus *Mesobuthus* (Scorpiones: Buthidae) in China, and described a new species: *Mesobuthus karshius* Sun & Sun, 2011. Recently, Kovařík (2012a) reported five new species of genus *Chaerilus*, including one new species from China: *Chaerilus wrzecionkoi* Kovařík, 2012. Di et al (2013a) reviewed the scorpions from Hainan Island and listed five species belonging to four genera of two families. Di & Fet (2012, personal communication) questioned the validity of *Mesobuthus martensii hainanensis* in Hainan. Di et al (2013b) analysed the scorpions from Tibet: 26 species of seven genera of five families were recorded in Tibet, all with distribution in south and the north shore of Yarlung Zangbo Jiang.

Given both the earlier literature and the most recent and detailed study, the total count for scorpions in China includes 53 species of 12 genera of five families. Particularly worth noting at four scorpion genera with higher diversity in China than elsewhere: *Chaerilus* (8), *Euscorpiops* (11), *Mesobuthus* (9) and *Scorpiops* (11) with more species.

## Taxonomy

Phylum: Arthropoda

Subphylum: Chelicerata

Class: Arachnida

Order: Scorpionida

## Family Buthidae C. L. Koch, 1837

Buthidae: Fet & Lowe, 2000: 54–57; Soleglad & Fet, 2003: 89–91.

Chinese members: six genera, 18 species and subspecies.

Distribution in China (provinces): most areas of China, except Guangdong, Guizhou, Heilongjiang, Hunan, Jiangxi, Jilin, Sichuan, Zhejiang.

## Genus *Hottentotta* Birula, 1908

*Hottentotta*: Fet & Lowe, 2000: 134–135; Kovařík, 2007: 2–3, 8–10; Sun et al, 2010: 40.

Chinese members: two species.

Distribution in China: Tibet.

### 1. *Hottentotta alticola* (Pocock, 1895)

*Hottentotta* (*Hottentotta*) *alticola minusalta*: Fet & Lowe, 2000: 135.

*Hottentotta* (*Hottentotta*) *alticola nigrifrons*: Fet & Lowe, 2000: 136.

*Hottentotta alticola*: Kovařík, 2007: 10, figs. 21–22; Zhu et al, 2004: 111.

Distribution in China: Unknown.

### 2. *Hottentotta songi* (Lourenço, Qi & Zhu, 2005)

*Mesobuthus songi* Lourenço, Qi & Zhu, 2005: 3–8, figs. 1–17, tab. 1.

*Hottentotta songi*: Teruel & Rein, 2010: 7; Sun et al, 2010: 40–42, figs. 25–29.

Distribution: Tibet (endemic (unique to China)).

### Genus *Isometrus* Ehrenberg, 1828

*Isometrus*: Thorell, 1876: 8; Sissom, 1990: 101; Fet & Lowe, 2000: 146; Kovařík, 2003: 1–2.

Chinese members: two subgenera, three species.

Distribution in China: Hainan, Taiwan, Tibet.

### Subgenus *Isometrus* Ehrenberg, 1829

*Isometrus* (*Isometrus*): Fet & Lowe, 2000: 146; Kovařík, 2003: 2.

Chinese members: one species.

Distribution in China: Hainan, Taiwan, Tibet.

### 3. *Isometrus* (*Isometrus*) *maculatus* (DeGeer, 1778)

*Isometrus* (*Isometrus*) *maculatus*: Fet & Lowe, 2000: 147; Kovařík, 2003: 2–4; Zhu et al, 2004: 112.

Di et al, 2013a: 4, 7, figs. 1–29, tabs. 1–2.

*Isometrus* (*Raddyanus*) *europaeus*: Tikader & Bastawade, 1983: 286–292, figs. 824–840.

Distribution: Hainan, Taiwan.

### Subgenus *Reddyanus* Vachon, 1972

*Isometrus* (*Reddyanus*): Fet & Lowe, 2000: 151; Kovařík, 2003: 5.

Chinese members: two species.

Distribution in China: Hainan, Tibet.

### 4. *Isometrus* (*Raddyanus*) *hainanensis* Lourenço, Qi & Zhu, 2005

*Isometrus* (*Reddyanus*) *hainanensis* Lourenço, Qi & Zhu, 2005: 58, 60, figs. 1–15, 17–19, tab. 1.

Distribution in China: Hainan (endemic).

### 5. *Isometrus* (*Reddyanus*) *tibetanus* Zhu & Lourenço, 2008

*Isometrus* (*Reddyanus*) *tibetanus* Zhu & Lourenço, 2008: 268–270, figs. 14–26, 32, tab. 1.

Distribution in China: Tibet (endemic).

### Genus *Lychas* C. L. Koch, 1845

*Lychas*: Tikader & Bastawade, 1983: 40; Kovařík, 1997: 312–314.

Chinese members: two species.

Distribution in China: Guangxi, Hainan, Shanghai (?), Yunnan.

### 6. *Lychas mucronatus* (Fabricius, 1798)

*Lychas mucronatus*: Kovařík, 1997: 341–344, figs. 10, 12, 29, 31, 80–82, 93, 98; Fet & Lowe, 2000: 164–165; Zhu et al, 2004: 112; Di et al, 2013a: 7, 12, 15, figs. 48–69, tabs. 1–2.

Distribution in China: Guangxi, Hainan, Yunnan.

#### **7. *Lychas scutillus* C. L. Koch, 1845**

*Lychas scutillus*: Kovařík, 1997: 351–352, figs. 41, 47–76, tabs. 1–3; Fet & Lowe, 2000: 166; Zhu et al, 2004: 112.

Distribution in China: Shanghai?

Comments: Fet et al (2000) recorded this species' distribution in China, but they questioned its authenticity. Kovařík & Whitman (2004) reported *Lychas scutillus* C.L. Koch, 1845 in China, and provided the information: “1 ♀ (591), Cina: Shanghai, 1878, d. [G.] Branchi. Note: È la prima segnalazione per la Cina”.

#### **Genus *Mesobuthus* Vachon, 1950**

*Mesobuthus* Vachon 1950: Sun, Zhu & Lourenço, 2010: 35.

Chinese members: nine species and subspecies.

Distribution in China: the north of the Changjiang River (except Heilongjiang and Jilin), Hainan (?), Shanghai (?).

#### **8. *Mesobuthus bolensis* Sun, Zhu & Lourenço, 2010**

*Mesobuthus bolensis* Sun, Zhu & Lourenço, 2010: 36, 40, figs. 2–3, 5–11, 14–18, 21–22, tab. 1; Sun & Sun, 2011: 59–60.

Distribution in China: Xinjiang (endemic).

#### **9. *Mesobuthus caucasicus intermedius* (Birula, 1897)**

*Mesobuthus caucasicus intermedius*: Shi & Zhang, 2005: 475; Sun & Zhu, 2010b: 3–4, 7–8, figs. 2, 11–13; Sun & Sun, 2011: 61–63, figs. 3–4, tab.1.

*Olivierus caucasicus intermedius*: Fet & Lowe, 2000:191; Zhu et al, 2004:113.

Distribution in China: Xinjiang.

#### **10. *Mesobuthus caucasicus przewalskii* (Birula, 1897)**

*Mesobuthus caucasicus przewalskii*: Shi & Zhang, 2005: 475; Sun & Zhu, 2010b: 4–5, 7–8, figs. 3, 14–16; Sun & Sun, 2011: 60–61, figs. 1–2, tab. 1.

*Olivierus caucasicus przewalskii* (Birula): Fet & Lowe, 2000: 192; Zhu et al, 2004: 113.

Distribution in China: Xinjiang.

#### **11. *Mesobuthus eupeus mongolicus* (Birula, 1911)**

*Mesobuthus eupeus mongolicus*: Sun & Sun, 2011: 67–70, figs. 7–8, tab. 1; Zhu et al, 2004: 112.

Distribution in China: Gansu, Inner Mongolia (Neimenggu), Ningxia.

#### **12. *Mesobuthus eupeus thersites* (C. L. Koch, 1839)**

*Mesobuthus eupeus thersites*: Sun & Sun, 2011: 70–72, tab. 1; Zhu et al, 2004: 113.

Distribution in China: Xinjiang.

#### **13. *Mesobuthus karshius* Sun & Sun, 2011**

*Mesobuthus karshius*: Sun & Sun, 2011: 63–67, figs. 5–6, tab.1.

Distribution in China: Xinjiang (endemic).

#### **14. *Mesobuthus longichelus* Sun & Zhu, 2010**

*Mesobuthus longichelus* Sun & Zhu, 2010b: 5–10, figs. 1, 4–10, 17–21.

Distribution in China: Xinjiang (endemic).

**15. *Mesobuthus martensii martensii* (Karsch, 1879)**

*Buthus martensi* Karsch: Kraepelin, 1899: 25–26; Wu, 1936: 115–117, fig. 1; Song et al, 1982: 22–25, figs. 1–7; Song, 1998: 508, fig. 30: 1.

*Mesobuthus martensii* (Karsch): Kovařík, 1998: 115; Shi & Zhang, 2005: 474; Shi et al, 2007: 216–223, figs. 1–3, tab. 1; Zhang & Zhu, 2009: 1–17, tabs. 1–8; Sun & Zhu, 2010b: 10.

*Mesobuthus martensii martensii* (Karsch): Fet & Lowe, 2000: 178; Qi et al, 2004: 137–143, figs. 1–19, tab. 1; Zhu et al, 2004: 113; Sun & Sun, 2011, 72, fig. 9, tab. 1.

Distribution in China: Shi et al (2007) summarized the distribution range of *M. martensii* (Karsch, 1879) in China as the south side of N43° and the north side of the Yangtze River, bordered by the Helan Mountains and the Tengger and Mo Us sand desert in the west and limited by the sea in the east. Kovařík & Whitman (2004) reported *Mesobuthus martensii* (Karsch, 1879) distributed in Shanghai, and the simple information as follows: “2♀♀ (592), Cina: Shanghai, 1878, d. [G.] Branchi”.

**16. *Mesobuthus martensii hainanensis* (Birula, 1904)**

*Buthus confucius hainanensis* Birula, 1904: 27; Sun & Sun, 2011: 72.

*Mesobuthus martensii hainanensis*: Fet et al, 2000: 178; Zhu et al, 2004: 113.

Distribution in China: Hainan (endemic?).

Comments: Di & Fet questioned the facticity of its distribution in Hainan (2012, personal communication). Birula (1904) described this subspecies, but did not provide a detailed description or illustrations; he did not report the gender or discuss relationship between this species and nominotypic *M. martensii*. We have not found any *Mesobuthus* species in Hainan, and we question the authenticity of this record. Birula (1904) studied specimens labeled “Hainan” and collected by Alfred Otto Herz (St. Petersburg, Russia) who conducted entomological expeditions in the 1890s to China, Korea, Japan, and Siam. Herz did indeed visit Hainan; his collections of reptiles and insects from Hainan were among the first ever made by Europeans. Therefore, *M. martensii hainanensis* could be a case of a mistaken label. Shi et al (2007) confirmed the distribution range of *M. martensii* in China by extensive field surveys and predictive models, and considered this species restricted to latitudes south of N43° and the north side of the Yangtze River, bordered by the Helan Mountains and the Tengger and Mo Us sand desert in the west and limited by the sea in the east.

**Genus *Orthochirus* Karsch, 1891**

*Orthochirus*: Kovařík, 2004c; Lourenço & Leguin, 2011a & b.

Chinese members: one species.

Distribution in China: general northwest.

**17. *Orthochirus scrobiculosus* (Grube, 1873)**

*Orthochirus scrobiculosus*: Shi & Zhang, 2005: 475.

Distribution in China: general northwest.

**Genus *Razianus* Farzanpay, 1987**

*Razianus*: Lourenço et al, 2010: 307–308.

Chinese members: one species.

Distribution in China: Xinjiang.

**18. *Razianus xinjianganus* Lourenço, Sun & Zhu, 2010**

*Razianus xinjianganus* Lourenço, Sun & Zhu, 2010: 308–309, 311–312, figs. 1–2, tab. 1.

Distribution in China: Xinjiang (endemic).

**Family Chaerilidae Pocock, 1893**

Chaerilidae: Fet, 2000a: 323. Kovařík, 2000a: 40–41; Soleglad & Fet, 2003: 92.

Chinese members: one genus, eight species.

Distribution in China: Tibet.

**Genus *Chaerilus* Simon, 1877**

*Chaerilus*: Fet, 2000: 323; Kovařík, 2000a: 38; Kovařík, 2005: 1; Qi et al, 2005: 29; Lourenço & Zhu, 2008: 462.

Chinese members: eight species.

Distribution in China: Tibet.

**19. *Chaerilus conchiformis* Zhu, Han & Lourenço, 2008**

*Chaerilus pictus*: Qi et al, 2005: 34, 38, figs. 126–144.

*Chaerilus conchiformis* Zhu, Han & Lourenço, 2008: 38–42, figs. 1–29, tab. 1.

Distribution in China: Tibet (endemic).

**20. *Chaerilus dibangvalleyicus* Bastawade, 2006**

*Chaerilus dibangvalleyicus* Bastawade, 2006: figs. 1–16.

Distribution in China: Tibet (endemic).

**21. *Chaerilus mainlingensis* Di & Zhu, 2009**

*Chaerilus mainglingensis* Di & Zhu, 2009b: 97–98, 101, figs. 1–16.

Distribution in China: Tibet (endemic).

**22. *Chaerilus pictus* (Pocock, 1890)**

*Chaerilus pictus*: Fet, 2000: 327; Kovařík, 2000a: 53–54; figs. 21–22, 39, 42–43, tabs. 1–2; Zhu et al, 2004: 113–114.

Distribution in China: Tibet.

**23. *Chaerilus tessellatus* Qi, Zhu & Lourenço, 2005**

*Chaerilus tessellatus* Qi, Zhu & Lourenço, 2005: 30, 34, figs. 109–125; Zhu et al, 2008: 44, 47, figs. 30–44, tab. 1.

Distribution in China: Tibet (endemic).

**24. *Chaerilus tricostatus* Pocock, 1899**

*Chaerilus tricostatus*: Fet, 2000a: 327; Kovařík, 2000a: 61–62, figs. 27–28, tabs. 1–2; Di et al, 2009: 133, 136–137, figs. 1–18, tab. 1.

Distribution in China: Tibet.

**25. *Chaerilus tryznai* Kovařík, 2000**

*Chaerilus tryznai* Kovařík, 2000a: 65–66, figs. 32–33, tabs. 1–2.

*Chaerilus tryznai*: Zhu et al, 2008: 47–48, 50–51, figs. 45–59, tab. 1.

Distribution in China: Tibet (endemic).

**26. *Chaerilus wrzecionkoi* Kovařík, 2012**

*Chaerilus wrzecionkoi* Kovařík, 2012b: 11, 13, figs. 62–77.

Distribution: Tibet (endemic).

**Family Euscorpiidae Laurie, 1896**

Euscorpiidae: Fet & Sissom, 2000: 355; Soleglad & Fet, 2003: 105.

Scorpionidae: Fet, 2000c: 487; Kovařík, 2000b: 154.

Chinese members: two genera, 22 species.

Distribution in China: Yunnan, Tibet.

**Genus *Euscorpiops* Vachon, 1980**

*Euscorpiops*: Fet & Sissom, 2000: 488; Kovařík, 2000b: 154; Kovařík, 2005: 1, 4; Kovařík, 2012a: 1, 3.

Chinese members: 11 species.

Distribution in China: Yunnan, Tibet.

**27. *Euscorpiops asthenurus* (Pocock, 1900)**

*Euscorpiops asthenurus*: Fet & Sissom, 2000: 488.

*Scorpiops asthenurus*: Kovařík, 2000b: 167, figs. 15, 28, 31, tabs. 1–3.

Distribution in China: Tibet.

**28. *Euscorpiops kamengensis* Bastawade, 2006**

*Euscorpiops kamengensis* Bastawade, 2006: 454, 456–457, figs. 17–26.

Distribution in China: Tibet (endemic).

**29. *Euscorpiops karschi* Qi, Zhu & Lourenço, 2005**

*Euscorpiops karschi* Qi, Zhu & Lourenço, 2005: 25, figs. 94–108; Di & Zhu, 2009c: 11, 14–15, figs. 1–27, tab. 1.

Distribution in China: Tibet (endemic).

**30. *Euscorpiops kubani* Kovařík, 2004**

*Euscorpiops kubani* Kovařík, 2004a: 14–16, figs. 1–6, tab. 1.

*Euscorpiops kubani*: Di et al, 2011a: 5–9, figs. 10–28, tabs. 1–2.

Distribution in China: Yunnan.

**31. *Euscorpiops novaki* Kovařík, 2005**

*Euscorpiops novaki* Kovařík, 2005: 4, 6, figs. 8, 11, 15–16, tab. 1.

Distribution in China: Tibet (endemic).

**32. *Euscorpiops puerensis* Di, Wu, Cao, Xiao & Li, 2010**

*Euscorpiops puerensis* Di et al, 2010b: 49–52, 54, 56, 58–59, figs. 1–34, tabs. 1–2.

*Euscorpiops puerensis*: Di et al, 2011a: 9, 12–15, figs. 29–49.

Distribution in China: Yunnan (endemic).

**33. *Euscorpiops shidian* Qi, Zhu & Lourenço, 2005**

*Euscorpiops shidian* Qi, Zhu & Lourenço, 2005: 18, 22, 25, figs. 78–93.

*Euscorpiops shidian*: Di et al, 2011a: 9, 15–19, figs. 50–68, tabs. 1–2.

Distribution in China: Yunnan (endemic).

**34. *Euscorpiops vachoni* Qi, Zhu & Lourenço, 2005**

*Euscorpiops vachoni* Qi, Zhu & Lourenço, 2005: 18, figs. 62–77.

*Euscorpiops vachoni*: Di et al, 2011a: 19–21, figs. 69–72.

Distribution in China: Yunnan (endemic).

**35. *Euscorpiops validus* Di, Cao, Wu & Li, 2010**

*Euscorpiops validus* Di et al, 2010: 14–17, 19, 21, figs. 1–32, tabs. 1–2.

*Euscorpiops validus* Di et al, 2011: 21, figs. 73–91.

Distribution in China: Yunnan (endemic).

**36. *Euscorpiops xui* Sun & Zhu, 2010**

*Euscorpiops xui* Sun & Zhu, 2010: 62, 67, figs. 1–14, tab. 1.

*Euscorpiops xui*: Di et al, 2011a: 21–25, figs. 92–110, tabs. 1–2.

Distribution in China: Yunnan (endemic).

**37. *Euscorpiops yangi* Zhu, Zhang & Lourenço, 2007**

*Euscorpiops yangi* Zhu et al, 2007: 20–22, 25, figs. 1–22, tab. 1.

*Euscorpiops yangi*: Di et al, 2011a: 26–28, figs. 111–117.

Distribution in China: Yunnan (endemic).

**Genus *Scorpiops* Peters, 1861**

*Scorpiops*: Fet & Sissom, 2000: 491; Kovařík, 2000b: 162, 164, 166; Qi et al, 2005: 2; Di & Zhu, 2009a: 40; Di et al, 2011b, 1–2. Kovařík, 2009: 1.

Chinese members: 11 species.

Distribution in China: Hubei, Yunnan, Tibet.

**38. *Scorpiops atomatus* Qi, Zhu & Lourenço, 2005**

*Scorpiops atomatus* Qi, Zhu & Lourenço, 2005: 6, 10, figs. 16–31.

Distribution in China: Tibet (endemic).

**39. *Scorpiops hardwickii* (Gervais, 1843)**

*Scorpiops hardwickii*: Kovařík, 2000b: 175–179, figs. 14, 46, 56–57.

*Scorpiops hardwickii hardwickii*: Fet & Sissom, 2000: 492.

Distribution in China: Tibet.

**40. *Scorpiops jendeki* Kovařík, 1994**

*Scorpiops hardwickii jendeki* Kovařík, 1994: 62, figs. 7–13, tab. 1; Fet, 2000: 492.

*Scorpiops jendeki*: Kovařík, 2000b: 180, 182, figs. 59–60, tabs. 1–3; Di et al, 2013b: 90, 93–94, figs. 119–135, tab. 3.

Distribution in China: Yunnan (endemic).

**41. *Scorpiops langxian* Qi, Zhu & Lourenço, 2005**

*Scorpiops langxian* Qi, Zhu & Lourenço, 2005: 10, 14, figs. 32–46.

Distribution: Tibet (endemic).

**42. *Scorpiops leptochirus* Pocock, 1893**

*Scorpiops leptochirus* Pocock, 1893: Fet & Sissom, 2000: 493.

Distribution: Tibet.

**43. *Scorpiops lhasa* Di & Zhu, 2009**

*Scorpiops lhasa* Di & Zhu, 2009a: 40–41, 45, 47, figs. 1–33, tab. 1.

Distribution: Tibet (endemic).

**44. *Scorpiops luridus* Qi, Zhu & Lourenço, 2005**

*Scorpiops luridus* Qi, Zhu & Lourenço, 2005: 2, 6, figs. 1–15.

Distribution: Tibet (endemic).

**45. *Scorpiops margerisonae* Kovařík, 2000**

*Scorpiops margerisonae* Kovařík, 2000b: 189, figs. 66, 70, tabs. 1–3; Di & Zhu, 2010: 1–8, figs. 1–23, tabs. 1–2.

Distribution: Tibet (endemic).

**46. *Scorpiops petersii* Pocock, 1893**

*Scorpiops petersii*: Kovařík, 2000b: 192–194, figs. 35, 42, tabs. 1–3; Fet & Sissom, 2000: 494.

Distribution: Tibet.

**47. *Scorpiops pococki* Qi, Zhu & Lourenço, 2005**

*Scorpiops pococki* Qi, Zhu & Lourenço, 2005: 14, figs. 47–61.

Distribution: Tibet (endemic).

**48. *Scorpiops tibetanus* Hirst, 1911**

*Scorpiops tibetanus* Hirst, 1911: 472–473; Kovařík, 2000b: 197, figs. 47, 68–69, tab.1–3; Fet & Sissom, 2000: 495; Di et al, 2013b: 75, 77, 80–81, 83, 85, figs. 102–118, tab. 2.

Distribution: Tibet (endemic).

**Family Hemiscorpiidae Pocock, 1893**

Chinese members: two genera, two species.

Distribution in China: Hainan, Tibet.

**Genus *Liocheles* Sundevall, 1833**

*Liocheles*: Monod & Volschenk, 2004: 677.

Chinese members: one species.

Distribution in China: Hainan.

**49. *Liocheles australasiae* (Fabricius, 1775)**

*Hormurusaustra lasiae*: Wu, 1936: 121–123, fig. 4; Tikader & Bastawade, 1983: 501–505, figs. 1362–1375.

*Liocheles australasiae*: Monod & Volschenk, 2004: 677; Di et al, 2013a: 15–16, 19–21, figs. 70–88, tabs. 1–2.

*Liocheles australasiae australasiae*: Fet, 2000b: 397.

Distribution: Hainan.

**Genus *Tibetiomachus* Lourenço & Qi, 2006**

*Tibetiomachus* Lourenço & Qi, 2006: 291.

Chinese members: one species.

Distribution in China: Tibet.

**50. *Tibetiomachus himalayensis* Lourenço & Qi, 2006**

*Tibetiomachus himalayensis* Lourenço & Qi, 2006: 291, 293–294, figs. 1: 5–14, 2: 5–26.

Distribution: Tibet (endemic).

**Family Scorpionidae Latreille, 1802**

Scorpionidae: Fet, 2000c: 427–428; Soleglad & Fet, 2003: 113–114.

Chinese members: one genus, three species.

Distribution in China: Tibet.

**Genus *Heterometrus* Ehrenberg, 1828**

*Heterometrus*: Fet, 2000d: 431; Kovařík, 2004b: 2, 4; Lourenço et al, 2005: 9.

Chinese members: three species.

Distribution in China: Tibet (?)

**51. *Heterometrus longimanus* (Herbst, 1800)**

*Heterometrus petersii* (Thorell, 1876): Zhu et al, 2004: 114.

Distribution in China: some indeterminate parts.

## 52. *Heterometrus tibetanus* Lourenço, Qi & Zhu, 2005

*Heterometrus tibetanus* Lourenço, Qi & Zhu, 2005: 10–14, figs. 18–34, tab. 1.

Distribution: Tibet (endemic).

## 53. *Heterometrus petersii* (Thorell, 1876)

*Heterometrus petersii* (Thorell, 1876): Zhu et al, 2004: 114.

Distribution in China: some indeterminate parts.

**Key to scorpions from China** (Di & Zhu, 2010; Di et al, 2009, 2010, 2011a, 2011b, 2013a, 2013b; Monod & Volschenk, 2004; Kovařík, 1997, 2000a, 2000b, 2003, 2004a, 2004b, 2005, 2007, 2009, 2012a, 2012b; Prendini, 2000; Söleglad & Fet, 2003; Sun et al, 2010; Sun & Sun, 2011; Zhu & Lourenço, 2005):

1. Orthobothriotaxic pattern type A; ventral aspect of leg tarsus with multiple irregular rows of setae, no trace of spinules (*configuration* 2); dorsal edge of cheliceral movable finger with two basal denticles; hemispermatophore is *flagelliform* (**Buthidae**) ..... 2
1. Orthobothriotaxic pattern type B or C; ventral aspect of leg tarsus with or without irregular setal rows, spinules present medially; dorsal edge of cheliceral movable finger with a *single* basal denticle; hemi-spermatophore is either *fusiform* or *lamelliform* ..... 19
2. Tibial spurs absent on all legs (**Isometrus**) ..... 3
2. Tibial spurs present on legs III & IV ..... 5
3. Trichobothrium *db* of the fixed finger in a distal position in relation to the trichobothria *et* and *est*; the distance between external trichobothria of the femur, *e<sub>1</sub>* and *e<sub>2</sub>* being at least two to five times the distance between trichobothria *e<sub>1</sub>* and *d<sub>3</sub>* of the femur (**subgenus Isometrus**); telson with 2 granules on the ventral surface ..... *Isometrus maculatus*
3. Trichobothrium *db* in a basal position to *et*, situated between *et* and *est*; the distance between external trichobothria of the femur, *e<sub>1</sub>* and *e<sub>2</sub>* always less than two times the distance between *e<sub>1</sub>* and *d<sub>3</sub>* (**subgenus Reddyanus**) ..... 4
4. Telson with 5 granules on the ventral surface ..... *Isometrus hainanensis*
4. Telson with 2 granules on the ventral surface ..... *Isometrus tibetanus*
5. Telson with subaculear tooth (**Lychas**) ..... 6
5. Telson without subaculear tooth ..... 7
6. In adults, total length of males longer than females, metasomes of males notable elongated ..... *Lychas scutillus*
6. In adults, with similar body and metasome length in both sexes (or males small) ..... *Lychas mucronatus*
7. Metasome with punctuate (**Orthochirus**) ..... *Orthochirus scrobiculosus*
7. Metasome with granule or smooth ..... 8
8. In adults, with smaller body (usually shorter than 20mm), carinae of prosome and mesosome weak (**Razianus**) .....  
..... *Razianus xinjianganus*
8. Medium to large body length (over 30 mm), carinae of prosome and mesosome strong ..... 9
9. Ventrolateral carinae of fifth metasomal segment with all granules more or less equal in size and never lobate (**Hottentotta**) ..... 10
9. Ventrolateral carinae of Metasoma segment V formed of disjunct and unequal granules, often enlarged posteriorly (**Mesobuthus**) ..... 11
10. Movable finger of pedipalp-chela with distinct granules divided into 14–16 rows, pedipalps without intense setation ..... *Hottentotta alticola*
10. Movable finger of pedipalp-chela with distinct granules divided into 13 rows, body and pedipalps covered with very intense setation ..... *Hottentotta songi*

11. Ventrolateral carinae of segment V on metasoma with several markedly large and extroversive lobed granules .....	12
11. Ventrolateral carinae of segment V on metasoma without markedly large and extroversive lobed granules .....	14
12. Ventral carinae of segment II and III of metasoma gradually stronger posteriorly .....	13
12. Ventral carinae of segment II and III of metasoma not stronger posteriorly .....	<i>Mesobuthus longichelus</i>
13. Anterior margin of carapace with a very weak median concavity, chelae more robust .....	..... <i>Mesobuthus eupeus thersites</i>
13. Anterior margin of carapace with a very weak median projection or approximately straight, chelae relatively less robust .....	<i>Mesobuthus eupeus mongolicus</i>
14. Ventral surface of segment V on metasoma without brown pigment .....	15
14. Ventral surface of segment V on metasoma with markedly brown pigment .....	16
15. Surfaces of carapace with relatively dense small granules, tarsus of legs with two long longitudinal rows of setae positioned ventrally .....	<i>Mesobuthus bolensis</i>
15. Surfaces of carapace between median carinae almost smooth, but the external surfaces with comparatively dense small granules, tarsus of legs with two short longitudinal rows of setae positioned ventrally .....	<i>Mesobuthus karshius</i>
16. Dorsal surfaces of metasomal segments I–IV and each surface of segment V with irregular net-like dark pigmentation .....	17
16. Only surfaces of segment V on metasoma with irregular net-like dark pigmentation, dorsal surfaces of segments I–IV without net-like pigmentation ( <i>Mesobuthus martensii</i> (Karsch, 1879)) .....	18
17. Pectinal teeth number 20–25 in females and 26–30 in males; dentate margins of movable and fixed fingers with 12 and 11 oblique rows of granules respectively .....	<i>Mesobuthus caucasicus intermedius</i>
17. Pectinal teeth numbers 15–19 in females and 19–23 in males; dentate margins of movable and fixed fingers with 11 and 10 oblique rows of granules respectively .....	<i>Mesobuthus caucasicus przewalskii</i>
18. Distributed on the north side of the Yangtze River .....	<i>Mesobuthus martensii martensii</i>
18. Distributed on Hainan Island .....	<i>Mesobuthus martensii hainanensis</i>
19. Orthobothriotaxic pattern type B; sternum is type 1; hemispermatophore is fusiform ( <b>Chaerilidae: Chaerilus</b> ) .....	20
19. Orthobothriotaxic pattern type C; sternum is type 2; hemispermatophore is lamelliform .....	27
20. Movable finger of pedipalp with 7–8 rows of granules .....	21
20. Movable finger of pedipalp with 10–14 rows of granules .....	25
21. Chela length to width ratio in adults 1.6–1.8 .....	<i>Chaerilus conchiformus</i>
21. Chela length to width ratio in adults higher than 2.0 .....	22
22. Ventral side of seventh mesosomal segment with 2 pair of granular carina, anterior margin straight with a median notch .....	23
22. Ventral side of seventh mesosomal segment with many granules but without carina, anterior margin straight without median notch .....	24
23. Pedipalp femur short than carapace; 8–9 minute teeth on inner ventral margins of movable and immovable fingers respectively .....	<i>Chaerilus dibangvalleycus</i>
23. Pedipalp femur longer than carapace, 7–8 minute teeth on inner ventral margins of movable and immovable fingers respectively .....	<i>Chaerilus mainlingensis</i>
24. Manus of pedipalp in male narrow and long. Chela length/width ratio in male higher than 3 .....	..... <i>Chaerilus tryznai</i>
24. Manus of pedipalp in male robust (Fig. 68). Chela length/width ratio in adults lower than 2.6 .....	..... <i>Chaerilus wrzecionkoi</i>
25. Movable finger of pedipalp with 13–14 rows of granules; telson of male rather long and about 4.7 times greater length than width, with obvious sexual dimorphism .....	<i>Chaerilus pictus</i>
25. Movable finger of pedipalp with 11–12 rows of granules, telson of male and female without sexual dimorphism, manus lacks 1 dorsal carina .....	26
26. Carapace, tergites nearly smooth in adults, chelicerae dorsal aspect without granules .....	<i>Chaerilus tessellatus</i>
26. Carapace, tergites with many big granules in adults, chelicerae dorsal aspect with granules .....	<i>Chaerilus tricostatus</i>

27. Legs with two pedal spurs (though one or more pedal spurs are lost in many troglobitic species); ventral aspect of leg tarsus equipped with moderately developed setal pairs and/or median row of spinules (configuration 5); paraxial organ without reflection of internobasal sperm duct (**Chactoidae**, see Soleglad & Fet, 2003, p. 92–93: Key to the superfamilies of parvorder **Iurida**); chelal fingers equipped with inner accessory denticles (IAD), outer denticles (OD) situated outside of median denticle (MD) row; major variable neobothriotaxy present, types Eu1 and Eu2; chelal palm is flat in appearance, carinae D3 and V2 essentially obsolete, angle formed by carinae D3:D4:D5 greater than 90° (**Euscorpiidae**, see Soleglad & Fet, 2003, p. 94: Key to the families of superfamily **Chactoidae**) ..... 28
27. Legs with one pedal spur (retrolateral spur absent, though this character is reversed in some bothriurid genera); ventral aspect of leg tarsus equipped with pairs of large limbated socketed setae, median spinule row optional (configuration 4); paraxial organ with reflection of internobasal sperm duct (**Scorpionoidea**, see Soleglad & Fet, 2003, p. 92–93: Key to the superfamilies of parvorder **Iurida**) ..... 47
28. Tricho-bothrium  $Eb_3$  on external surface of chela is located between trichobothria Dt and Est. Telson vesicle/aculeus juncture with annular ring (**Euscorpiops**) ..... 29
28. Trichobothrium  $Eb_3$  on the external aspect of pedipalp chela located basally from trichobothrium Dt. Annular ring at vesicle/aculeus juncture absent (**Scorpiops**) ..... 39
29. Number of trichobothria on external surface of pedipalp patella: 19 (5 *eb*, 2 *esb*, 2 *em*, 5 *est*, 5 *et*) ..... 30
29. Number of trichobothria on external surface of pedipalp patella: 17–19 (5–6 *eb*, 1–2 *esb*, 2 *em*, 4 *est*, 5 *et*) ..... 32
30. Pattern of trichobothria on external surface of pedipalp patella: 19 (6 *eb*, 2 *esb*, 2 *em*, 4 *est*, 5 *et*) ..... *Euscorpiops xui*
30. Pattern of trichobothria on external surface of pedipalp patella: 19 (5 *eb*, 2 *esb*, 2 *em*, 5 *est*, 5 *et*) ..... 31
31. Number of trichobothria on ventral surface of patella: 7; number of pectinal teeth: 4–5; movable finger longer than carapace and as long as pedipalp femur ..... *Euscorpiops kamengensis*
31. Number of trichobothria on ventral surface of patella: 9; pectinal teeth number 8; movable finger as long as carapace and shorter than pedipalp femur ..... *Euscorpiops novaki*
32. Male pedipalp chela finers strongly scalloped: with a pronounced lobe on the movable finger and a corresponding notch on fixed finger ..... 33
32. Male pedipalp chela fingers slightly scalloped or straight: lobe and corresponding notch reduced to absent ..... 37
33. Chela length to width ratio higher than 2.9 ..... 34
33. Chela length to width ratio lower than 2.9 ..... 36
34. Female pedipalp fingers nearly straight ..... *Euscorpiops asthenurus*
34. Female pedipalp fingers obviously scalloped ..... 35
35. Chela length to width ratio 2.9–3.2, carapace with dense, minute granules, total length over than 50 mm, coloration basically dark brown ..... *Euscorpiops validus*
35. Chela length to width ratio 3.4–3.5, carapace with sparse, nearly equal granules, total length less than 50 mm (small species), coloration basically dark red-brown ..... *Euscorpiops karschi*
36. Chela manus short, stout and rounded ..... *Euscorpiops vachoni*
36. Chela manus flattened dorsoventrally ..... *Euscorpiops puerensis*
37. Chela length to width ratio higher than 3.2 ..... 38
37. Chela length to width ratio lower than 3.2 ..... *Euscorpiops kubani*
38. Number of trichobothria on ventral surface of patella: 11, chela length to carapace length ratio less than 1.4 ..... *Euscorpiops shidian*
38. Number of trichobothria on ventral surface of patella: 10, chela length to carapace length ratio higher than 1.9 ..... *Euscorpiops yangi*
39. Fingers of pedipalps are straight or only slightly flexed in both sexes ..... 40
39. Fingers of pedipalps are flexed (or curved) in both sexes ..... 41
40. Ventral trichobothria on patella number 6 (7 rarely), total length 30–42.1 mm, pectinal teeth number 4–5, chela length to width ratio about 2.2 ..... *Scorpiops jendeki*
40. Ventral trichobothria on patella number 7, total length 40–58 mm, pectinal teeth number 7–9, chela length to width ratio about 3.3–3.5 ..... *Scorpiops leptochirus*

41. Male chela length to width ratio about 1.8–2.2; the manus with same or very similar length and width, fingers of pedipalps are very strongly flexed in the male. Ventral trichobothria on patella number 6–8 ..... *Scorpiops hardwickii* “complex” group (including *S. hardwickii*, *S. langxian*, and *S. pococki*)
41. Manus length to width ratio visibly higher than 1.0 ..... 42
42. Total length more than 65 mm ..... 43
42. Total length less than 65 mm ..... 44
43. Mostly yellowish to yellow in adults, ventral patella of pedipalps with 9 trichobothria ..... *Scorpiops luridus*
43. Mostly red brown in adults, ventral patella of pedipalps with 7 (rarely 6 or 8) trichobothria ..... *Scorpiops petersii*
44. Dorsally flat manus of pedipalps and chela of both sexes with length/width ratio: 2.1–2.2 (mean about 2.1 in males and 2.2 in females), total length 40.0–50.0 mm in adults ..... *Scorpiops margerisonae*
44. Dorsally round manus of pedipalps or at least the chela of one sex with length to width ratio higher than 2.2 or total length higher than 50 mm ..... 45
45. Total length less than 40 mm ..... 46
45. Total length more than 50 mm, chela strong, with length/width ratio: 2.0 in male and 2.5 in female ..... *Scorpiops tibetanus*
46. Chela of pedipalp length to width ratio about 2.6–3.0, dorsal surface of chela of pedipalp coarse ..... *Scorpiops lhasa*
46. Chela of pedipalp length to width ratio lower than 2.5, dorsal surface of chela of pedipalp smooth with luster ..... *Scorpiops atomatus*
47. Median ocular tubercle of carapace shallow, not raised above carapace surface; 2 pairs of lateral eyes; telotarsus with lateral lobes truncated; *Est* located in middle of hand (**Hemiscorpiidae**, see Stockmann & Ythier, 2010, p. 201) ..... 48
47. Median ocular tubercle raised up; 3 pairs of lateral eyes; telotarsus with lateral margins ending in rounded lobes; *Est* located in distal of hand (**Scorpionidae**, see Stockmann & Ythier, 2010, p. 201); pedipalp femur with three trichobothria; patella of pedipalp with 19 trichobothria, three on ventral and 13 on external surface; chela of pedipalp with 26 trichobothria; retrolateral pedal spurs absent; lateroapical margins of tarsi produced into rounded lobes; metasomal segments I to IV with paired ventral submedian carinae; stridulatory organ located on opposing surfaces of pedipalp coxa and first leg; total length 60 to 180 mm (**Heterometrus**, see Kovařík, 2004, p. 2) ..... 49
48. Chela trichobothrium *dt* present (**Liocheles**) ..... *Liocheles australasiae*
48. Chela trichobothrium *dt* absent (**Tibetiomachus**) ..... *Tibetiomachus himalayensis*
49. Chela round and strong ..... *Heterometrus petersii*
49. Chela narrow and long ..... 50
50. Chela manus with weak granules on the dorso-internal aspect ..... *Heterometrus longimanus*
50. Chela manus with strong spinoid granules on the dorso-internal aspect ..... *Heterometrus tibetanus*

## DISCUSSION

The distribution of scorpions is quite well demarcated by very distinct features arising from the geography and topography of China: there are markedly different constituents between east and west, north and south. Only four recorded species can be found living in both East China and Chinese islands (based on recently finished investigations): *Isometrus maculatus* (DeGeer, 1778), *Lychas mucronatus* (Fabricius, 1798), *Liocheles australasiae* (Fabricius, 1775), and *Mesobuthus martensii martensii* (Karsch, 1879); Three species maybe extinct or invalid: *Isometrus hainanensis* Lourenço, Qi & Zhu,

2005, *Lychas scutillus* C. L. Koch, 1845, and *Mesobuthus martensii hainanensis* (Birula, 1904). In Central China, only one species is reported: *Mesobuthus martensii martensii* (Karsch, 1879).

All of the species of genera *Chaerilus*, *Euscorpiops*, *Scorpiops*, and most of *Mesobuthus* live in the west of China, which is unsurprising as Tibet, Yunnan, and Xinjiang are the richest areas of scorpion biodiversity in all of China. Meanwhile, there are only nine species of two genera reported in northern China (including Xinjiang): *Mesobuthus* (eight species) and *Razianus* (one species).

Zhu et al (2004) published a list of Chinese scorpions based on the information of Fet et al (2000) and Kishida (1939), which included 5 families, 9 genera, 19 species and subspecies. Qi et al (2005) discovered eight new species of Chaerilidae (*Chaerilus*) and Euscorpiidae (*Euscorpiops* and *Scorpiops*). Di et al (2011a) reviewed the scorpions of Yunnan, recording eight previously known species and described one new species to China: *Euscorpiops kubani* Kovařík, 2004. Sun & Sun (2011) reviewed the genus *Mesobuthus* (Scorpiones: Buthidae) in China, recorded Nine species and subspecies including a new species: *Mesobuthus karshius* Sun & Sun, 2011. Di et al (2013a, b) reviewed the scorpions from Hainan Island and Tibet: 5 species and 26 species were recorded in Hainan and Tibet respectively.

While not completely exhaustive, this study nonetheless gives an excellent overview on the

general situation of scorpion biodiversity in China. At present, 53 scorpion species of 12 genera of 5 families are recorded in China, which has greatly helped clarify the characters of biodiversity and distribution of scorpions. This overview—including a history of taxonomic research, an updated checklist and key of the scorpions of China—accordingly is aimed at producing a catalogue of scorpions in China. While the survey on scorpion species diversity in China is not completed yet, we hope that this overview can provide researchers in both mainland China and abroad with enough basic data for taxonomy, fauna, and resources to help further their efforts.

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# Morphological and molecular studies on *Garra imberba* and its related species in China

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**Abstract:** *Garra imberba* is widely distributed in China. At the moment, both *Garra yiliangensis* and *G. hainanensis* are treated as valid species, but they were initially named as a subspecies of *G. pingi*, a junior synonym of *G. imberba*. *Garra alticorpora* and *G. nujiangensis* also have similar morphological characters to *G. imberba*, but the taxonomic statuses and phylogenetic relationships of these species with *G. imberba* remains uncertain. In this study, 128 samples from the Jinshajiang, Red, Nanpanjiang, Lancangjiang, Nujiang Rivers as well as Hainan Island were measured while 1 mitochondrial gene and 1 nuclear intron of 24 samples were sequenced to explore the phylogenetic relationship of these five species. The results showed that *G. hainanensis*, *G. yiliangensis*, *G. alticorpora* and *G. imberba* are the same species with *G. imberba* being the valid species name, while *G. nujiangensis* is a valid species in and of itself.

**Keywords:** *Garra imberba*; Taxonomy; Morphology; Molecular phylogeny

With 105 valid species *Garra* is one of the most diverse genera of the Labeoninae, and has a widespread distribution ranging from East Asia to Africa (Froese & Pauly, 2012). Menon (1964) first revised the genus and divided it into 4 groups and 9 complexes. *Garra imberbis* and *G. imberba* were classified into the *imberbis* complex, which is distinguished from the other complexes by having more lateral line scales, a larger body size, a shorter distance between vent and the pelvic fin base, and no barbels (Menon, 1964).

Garman (1912) described *Garra (Ageneiogarra) imberba* using samples from the Min River, Kiating, Szechuan (Leshan, Sichuan), China. *G. imberba* can be distinguished from other *Garra* species by its lack of barbels, 46–52 lateral line scales, 16 circumpeduncle scales, no secondary rostrum, and an anus close to pelvic-fin base. *Ageneiogarra* was treated as a synonym of *Garra*, because the number of barbels was thought to be not eligible to define a new subgenus during the initial intensive study of cyprinid fish. (Karaman, 1971; Kottelat, 1998). Fang (1943) later renamed the materials

from Jinshajiang River as *G. pingi*, but Kottelat (1998) treated *G. imberba* as a valid name, and this convention is still widely accepted at present.

Four subspecies of *Garra imberba* have also been identified, although their species validity is still uncertain. Wu et al (1977) described a new subspecies, *G. pingi yiliangensis*, based on the materials from Nanpanjiang River. *G. pingi yiliangensis* has 9 branched dorsal-fin rays, a standard length 5.7–6.1 times body depth, and the caudal-peduncle is 2.0–2.1 times longer than its depth. Zheng & Chen (1983) described another subspecies from Hainan Island, named *G. pingi hainanensis*. It has 46–47 lateral-line scales, and the length of caudal-peduncle is 1.1–1.2 times its depth. *Garra imberba*, *G. pingi yiliangensis*, and *G. pingi hainanensis* all have isolated

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distributions, so they were treated as distinct species under the phylogenetic species as conceptualized by Kottelat (1998). *Garra pingi* caught from the Nujiang River (upper Salween River) were later described as *G. nujiangensis* by Chen et al (2009). It has 12–14 circumpeduncle scales, and some of its characteristics are meristically and metrically different from *G. imberba*. However, Chen et al also found that the new species has some characteristics similar with *G. imberba*, especially among juveniles, so they just temporarily treated it as a distinct species from *G. imberba*. Lastly, *Garra alticorpora* was named by Chu & Cui (1987) based on two specimens from Pingbian, Yunnan, which belongs to the Red River Drainage system. The most significant characteristic of this *Garra* is that its body depth is longer (vs. shorter) than its head length. After original sampling, however, specimens collected from the same location did not have the same characteristics, so the two *G. alticorpora* samples were thought to be gravid females and treated as a synonym of *G. imberba* by Zhou et al (2005). Besides *G. imberba*, all four of these species (*G. pingi yiliangensis*, *G. pingi hainanensis*, *G. nujiangensis*, *G. alticorpora*) were known as either subspecies of it or considered synonym of it, and their species validity and phylogenetic relationship is still uncertain.

The mitochondrial DNA and nuclear gene have been widely used in the studies of molecular phylogenetic analyses of subfamily Labeoninae or genus *Garra* (He et al, 2008; Li et al, 2005; Tang et al, 2009; Yang & Mayden, 2010; Zheng et al, 2010; Yang et al, 2012). In this study, morphological and molecular methods were used to investigate the phylogenetic relationships among the five species or subspecies of *G. imberba* and its related species distributed in China.

## MATERIALS AND METHODS

### Sampling

A total of 128 specimens were measured for morphologic analysis, including 90 *Garra imberba* from Jinshajiang and Red-Mekong River, 9 *G. yiliangensis* from Nanpanjiang River, 11 *G. hainanensis* from Hainan Island, 16 *G. nujiangensis* from Nujiang River and 2 *G. alticorpora* from Red River. A total of 24 samples were used in molecular phylogenetic analyses. The collection localities are shown in Figure 1. *Garra yiliangensis* is only distributed in Nanpanjiang and Beipanjiang Rivers (upper Pearl River), but it was not included in this study for molecular analysis because no specimen has been caught in the last decade. No new *G. alticorpora*

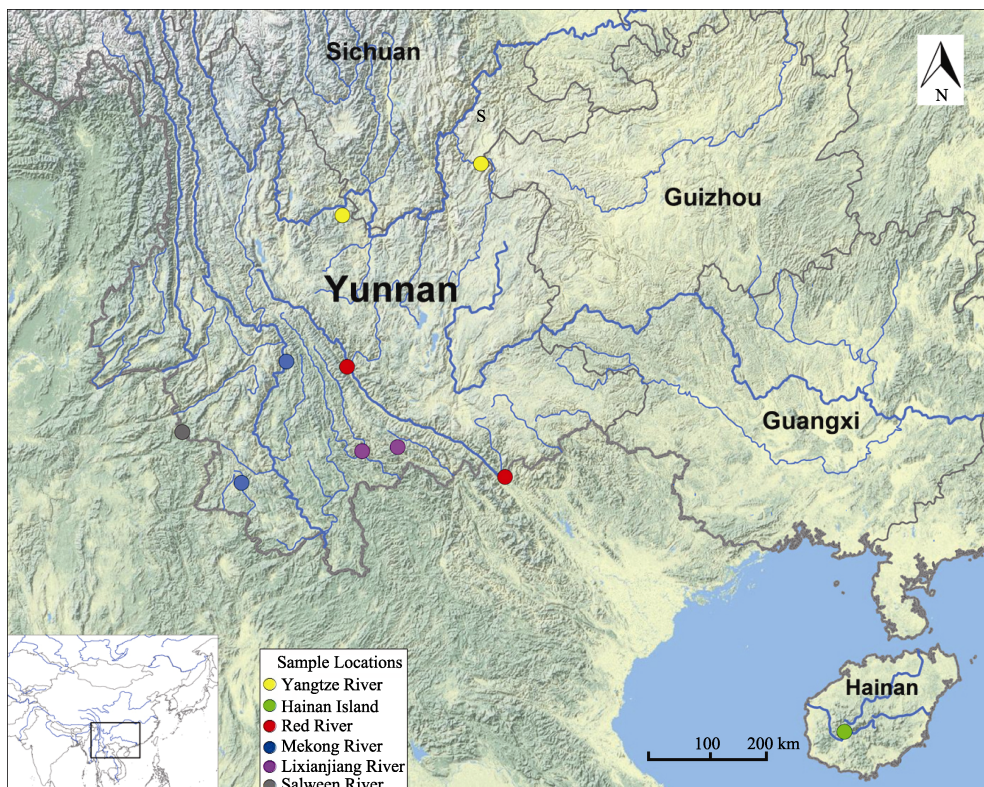


Figure 1 Geographic distribution of samples used in molecular analyses

specimens have been caught since it was named, so it was also not included in the molecular analysis. In addition, one sample of *Garra fasciacauda* from Pu'er, Yunnan was sequenced. This species was thought only to be distributed in the middle of the Mekong River, such as in Thailand and Cambodia, and this is the first record

of its presence in China.

All the specimens used in this study are deposited in the Collection Room of Fishes, Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS). Voucher information and GenBank accession numbers are listed in Table 1.

**Table 1 Taxa used in molecular analyses with source of each samples and GenBank accession numbers for each gene**

No.	Drainage	Voucher specimen	Taxon	Location	GenBank Accession No.	
					<i>cyt b</i>	S7
1	YZ1	KIZ041110093	<i>Garra imberba</i>	Chuxiong, Yunnan, China	KC119045	KC119071
2	YZ2	KIZ2010001891	<i>Garra imberba</i>	Huize, Yunnan, China	KC119046	KC119072
3	RR1	KIZ2010010001	<i>Garra imberba</i>	Xinping, Yunnan, China	KC119047	KC119073
4	RR2	KIZ2010010002	<i>Garra imberba</i>	Hekou, Yunnan, China	KC119048	KC119074
5	LX1	KIZ2004001193	<i>Garra imberba</i>	Puer, Yunnan, China	KC119049	KC119075
6	LX2	KIZ2009002070	<i>Garra imberba</i>	Lvchun, Yunnan, China	KC119050	KC119076
7	MK1	KIZ2008000088	<i>Garra imberba</i>	Xishuangbanna, Yunnan, China	KC119051	KC119077
8	MK2	KIZ2008008269	<i>Garra imberba</i>	Puer, Yunnan, China	KC119052	KC119078
9	HN1	KIZ2008003973	<i>Garra hainanensis</i>	Ledong, Hainan, China	KC119053	KC119079
10	HN2	KIZ2008003938	<i>Garra hainanensis</i>	Ledong, Hainan, China	KC119054	KC119080
11	SW1	KIZ2005005142	<i>Garra nuijiangensis</i>	Cangyuan, Yunnan, China	KC119055	KC119081
12	SW2	KIZ2005005145	<i>Garra nuijiangensis</i>	Cangyuan, Yunnan, China	KC119056	KC119082
13		KIZ2008005732	<i>Garra micropulvinus</i>	Wenshan, Yunnan, China	KC119057	KC119083
14		KIZ2011002800	<i>Garra findolabium</i>	Jinping, Yunnan, China	JQ864598	KC691274
15		KIZ5171	<i>Garra cryptonemus</i>	Liuku, Yunnan, China	JQ864587	KC691275
16		KIZ2007002789	<i>Garra caudofasciatus</i>	Jiangcheng, Yunnan, China	JQ864588	KC691276
17		KIZ2004014928	<i>Garra fasciacauda</i>	Puer, Yunnan, China	JQ864597	KC691277
18		KIZ2006004460	<i>Garra tengchongensis</i>	Tengchong, Yunnan, China	JQ864586	KC691278
19		KIZ2004000816	<i>Garra dulongensis</i>	Dulongjiang, Yunnan, China	JQ864590	KC691279
20		KIZ2006004422	<i>Garra qiaojiensis</i>	Yingjiang, Yunnan, China	JQ864583	KC691280
21		KIZ2006003543	<i>Garra salweenica</i>	Dehong, Yunnan, China	JQ864593	KC691281
22		KIZ2005000086	<i>Garra orientalis</i>	Longlin, Guangxi, China	JQ864581	KC691282
23		KIZ2005002392	<i>Garra mirofrontis</i>	Yunxian, Yunnan, China	JQ864584	KC691283
24			<i>Labeo senegalensis</i>		AB238968	AY103160

### Morphological analyses

Measurements were taken point to point with a digital caliper to the nearest 0.1 mm. Measurements and counts were made on the left side of individuals whenever possible (Kottelat, 2001). Predorsal, prepectoral, prepelvic and preanal lengths were taken respectively, from the anterior most tip of the snout to the dorsal-, pectoral-, pelvic- and anal-fin origins. Interorbital width

was measured between the upper margins of the eyes. Abbreviations used in this paper are as follows: SL (standard length), HL (head length), HD (head depth), HW (head width), DFL (dorsal fin length), PDL (predorsal fin length), PFL (pectoral fin length), PPL (prepectoral fin length), VFL (ventral fin length), PVL (preventral fin length), AFL (anal fin length), PAL (preanal fin length), CFL (caudal fin length), CPL (caudal

peduncle length), CPD (caudal peduncle depth), DAA (distance between anal and origin of anal fin), DVA (distance between ventral fin base and origin of anal fin), DL (disk length), DW (disk width), ED (eye diameter), IOW (interorbital width), SNL (snout length).

Principal component analysis (PCA) was used to examine the significance of difference among samples of different species or subspecies with SPSS 13.0. Default settings of factor analysis were applied for data standardization. Covariance matrix was used in the analysis. All samples were assigned into 5 groups based on species.

### DNA extraction and amplification

Total genomic DNA was extracted from the ethanol preserved specimens using a standard phenol/chloroform isolation and ethanol precipitation. Polymerase chain reaction (PCR) was used to amplify the sequences of one mitochondrial gene and one nuclear gene: cytochrome b (*cyt b*) and the first intron of S7 ribosomal protein (r-protein) gene. These genes are considered to be effective for understanding the evolutionary relationships among populations and species (He et al, 2008; Irwin et al, 1991). The complete *cyt b* gene was amplified with primers L14724 and H15915 (Xiao et al, 2001), S7 was amplified using primers S7RPEX1F and S7RPEX2R (Chow and Hazama, 1998). The PCRs contained approximately 100 ng of template DNA, 1 µL of each primer, 5 µL of 10× reaction buffer, 2 µL dNTPs (each 2.5 mM), and 1 U Taq DNA polymerase in total 50 µL volume. The PCR profile consisted of an initial denaturation step (3 min at 94 °C), followed by 35 cycles performed in the following order of denaturation at 94 °C for 1 min; annealing at 52 °C for 1 min; and elongation at 72 °C for 1 min; and a final extension at 72 °C for 8 min. PCR amplification products were purified using the Sangon DNA purification kit according to manufacturer's instructions. The purified PCR product was sequenced by the Shanghai Sangon Biological Engineering Technology & Services Co., Ltd.

### Sequence alignment and phylogenetic analyses

DNA sequences were edited using DNASTAR v7.1.0 (DNASTAR Inc.). All sequences were aligned by Clustal W and checked by eye in MEGA 5.0 (Tamura et al, 2011). Amino-acid translation was also conducted using MEGA 5.0 (Tamura et al, 2011) to confirm the correct reading frame positions and find unexpected stop

codons. The genetic distances (p-distance with 1 000 bootstrap) of the two sequences between taxa were calculated using MEGA 5.0 (Tamura et al, 2011). Two operational datasets were constructed for subsequent analyses: (i) mitochondrial DNA sequences, complete *cyt-b* (1 140 bp); (ii) nuclear DNA sequence, only include the first intron of S7 (805-809 bp).

Phylogenetic analyses were performed using maximum likelihood estimation (ML), and Bayesian inference methods with PAUP\* version 4.0 b10 (Swofford, 2003) and MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) respectively. For the ML analyses, a heuristic search was adopted with 1000 bootstrap replications, and a random tree was used as starting tree. Bayesian analyses was conducted for 5 million generations, using one cold and 3 incrementally heated chains and sampling every 100 generations. Two independent runs were performed to confirm convergence. Models were calculated by jModeltest v0.1.1 (Posada, 2008).

## RESULTS

### Morphological analyses

Counts and proportional measurements of *Garra imberba*, *G. yiliangensis*, *G. hainanensis*, *G. nujiangensis* and *G. alticorpora* are shown in Table 2. The factor analysis results showed that the first 3 components can explain 73.8% of the total variances. For the first 3 components, a scatter graph was drawn as follows (Figure 2). The 5 biggest correlations of the factor analyses of the first 3 components is shown in Table 3. Factor 1 reflects a primarily more slender body shape, and is composed mostly of samples of *G. yiliangensis* that are not clustered with the other studied species. There was no significant difference for factor 2 and 3 among these species.

### Molecular analyses

Among the two sequences used in this study, only *cyt b* is a protein coding gene and could be translated into amino acid sequences without interruption. The pairwise distances of *cyt b* and S7 are shown in Tables 4, 5 and 6. The overall mean p distance of clade A1 (*G. imberba* and *G. hainanensis*) based on *cyt b* and S7 are  $1.9\% \pm 0.3\%$  and  $0.2\% \pm 0.1\%$ . species of clade A3 contains valid species of *Garra* distributed in China and the mean distance within this clade based on *cyt b* and S7 are  $12.3\% \pm 0.5\%$  and  $11.2\% \pm 0.7\%$ . The significant difference within clade A1 and A3 implied that samples of clade A1 actually represent the same species.

Table 2 Counts and proportional measurements of *Garra imberba*, *G. yiliangensis*, *G. hainanensis* and *G. nuijiangensis*

	<i>n</i>	<i>G. imberba</i>						<i>G. yiliangensis</i>						<i>G. hainanensis</i>						<i>G. nuijiangensis</i>						<i>G. alticorpora</i>					
		90			9			11			16			2																	
		iv, 8-10	iv, 9-11	iv, 7-8	iv, 8-9	iv, 9		iv, 8-10	iv, 9-11	iv, 7-8	iv, 8-9	iv, 9		iv, 8-10	iv, 9-11	iv, 7-8	iv, 8-9	iv, 9		iv, 8-10	iv, 9-11	iv, 7-8	iv, 8-9	iv, 9		iv, 8-10	iv, 9-11	iv, 7-8	iv, 8-9	iv, 9	
Dorsal-fin rays		17.4	24.6	20.5	1.3	17	17.7	19.9	17.9	1	17.7	21.7	20.2	1.2	18.5	23.2	21.2	1.5	24.5	26.6	25.6	1.5									
Pectoral-fin rays		19.9	26.5	23.5	1.3	19.4	21	20.1	20.1	0.6	21.5	24.4	23.3	0.8	19.8	24	21.9	1.3	20.8	23.4	22.1	1.8									
Pelvic-fin rays		14.4	17.4	15.6	0.7	11.7	14	13	13	0.7	14.3	16.3	15.4	0.6	13	16.7	14.7	1.1	15.8	17	16.4	0.9									
Anal-fin rays		21.1	31.9	24.4	1.9	20.7	23.6	22.3	22.3	0.9	21.8	25.5	23.8	1.1	20.3	24.6	22.8	1.3	23.7	24	23.8	0.2									
Circumpeduncle scales		44.4	52.4	48.4	1.6	41.9	45.8	43.9	43.9	1.2	47.8	51.3	49.8	1.4	45.2	50.4	47.8	1.6	47.8	48.5	48.1	0.5									
Lateral-line scales		17.2	24.5	20.9	1.5	17	20.5	18.8	18.8	1.2	17.8	19.9	18.8	0.6	17.5	23.5	20.7	1.6	19.1	19.4	19.3	0.2									
In % of SL		19.6	27.9	22.7	1.4	18.9	20.5	19.8	19.8	0.6	20.9	23.7	22.2	0.9	18.8	22.3	20.4	1	20.6	21.2	20.9	0.5									
BD		16.4	23.9	19.3	1.3	16.5	19.6	18.4	18.4	0.9	15.9	18.4	17	0.7	17.6	20.5	19	0.9	18.4	19.5	18.9	0.8									
HL		48.6	57.2	52.5	2	45.6	53.3	49	49	2.4	49.5	54.4	51.7	1.5	50.2	56.3	52.6	1.4	53.3	53.3	53.3	0									
HD		15.7	22.3	18.7	1.4	16.1	18.7	17.4	17.4	0.8	15.7	18.6	17.1	0.8	16.4	19.5	17.7	0.8	18.6	18.7	18.7	0.1									
DFL		73.5	81.7	77.7	1.8	72.2	80.4	75.9	75.9	2.6	75	79.8	77.4	1.6	73.6	79.9	76.3	1.6	77.9	78.1	78	0.1									
PDPL		25.5	35.8	29.5	2.4	25.9	32.6	29.4	29.4	2.3	28.4	31.8	30.1	1	26.4	31.2	28.5	1.4	31.3	32.4	31.8	0.8									
PFL		11.9	19.2	15.5	1.5	14.5	20.9	18	18	2	14.7	16.7	15.8	0.6	15	18.5	16.5	0.9	16.3	16.5	16.4	0.1									
PPPL		8.7	12.9	10.9	0.7	8.5	10.5	9.2	9.2	0.8	9.7	11.1	10.6	0.4	10.1	11.8	10.9	0.4	11.7	13.2	12.5	1									
VFL																															
PVL																															
PFL																															
AFL																															
PAL																															
CFL																															
CPL																															
CPD																															
In % of HL																															
HW		66.6	88.2	76.9	4.3	75.7	85.2	79.6	79.6	3.2	69.8	79.2	75	2.8	73.7	85.9	78.5	3.2	86.8	89.6	88.2	2									
DL		34.8	49.7	40.8	2.8	36.1	45.7	40.3	40.3	3.4	35.7	41.6	39.2	1.7	33.9	42.5	38	2	39.2	43.5	41.3	3									
DW		45.5	72.3	57.6	5	55.9	67.7	59.6	59.6	4.3	47.1	59.9	52.3	3.5	50.7	56.3	52.8	1.6	61.8	53.9	57.8	5.6									
ED		18.1	28.7	22.3	2.3	18.4	23.7	20.3	20.3	1.7	18.7	21.4	19.6	0.8	20.3	24.4	22.1	1.4	19.7	20.2	19.9	0.3									
IOW		47	62.3	53	3.1	51	59.4	55.3	55.3	2.7	49.6	55.2	52.6	1.7	50.1	63.5	55.4	3	57	61.2	59.1	2.9									
SNL		41.5	56.9	50.5	2.7	53.1	58.3	55.8	55.8	1.6	51.4	55.1	53	1.3	47.2	57	52.4	2.6	54.9	57.1	56	1.5									
In % of CPL																															
CPD		55.8	93.2	71	7.6	42.9	72.3	51.9	51.9	9.8	64.4	74.9	67.3	3.2	57.5	77.2	66.3	5.5	70.9	81	75.9	7.2									
In % of DVA																															
DAA		75	86.6	80.9	2.5	79.9	83.8	82.1	82.1	1.6	78.8	84.5	82.2	1.7	75.6	82.7	79.2	2	77.9	81	79.4	2.2									

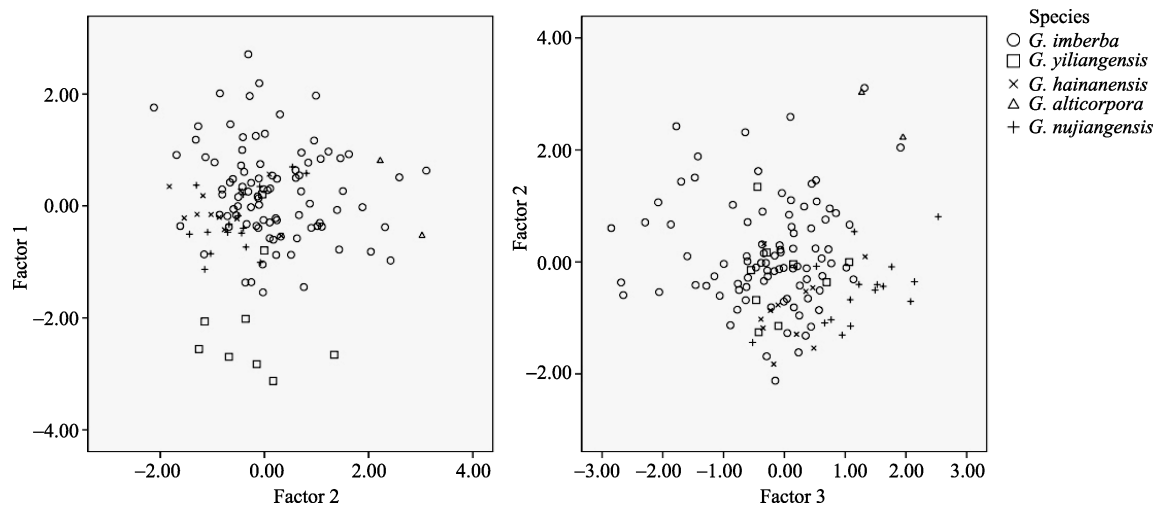


Figure 2 Scatter plots analyzed by PCA for samples from different locality groups based on 22 morphometric characters  
 A: scatter plot of scores on factor1 and factor 2; B: scatter plot of scores on factor2 and factor3; AL: *G. alticorpora*, from Red River; HN: *G. hainanensis*, from Hainan Island; LX: *G. imberba*, from Lixianjiang, the biggest tributary of Red river; MK: *G. imberba*, from Lancangjiang River; NP: *G. yiliangensis*, from Nanpanjiang River, the upper stream of Pear River; RR: *G. imberba*, from the main stream of Red River; SW: *G. nujiangensis*, from Nujiang River; YZ: *G. imberba*, from Jinshajiang River.

**Table 3 Proportion characteristics and principal components extracted from examined materials**

No.	Component 1	Correlation	Component 2	Correlation	Component 3	Correlation
1	0.966	CPD/CPL	0.842	DW/HL	0.648	IOW/HL
2	-0.859	CPL/SL	0.773	HW/HL	-0.457	DL/HL
3	0.648	HL/SL	0.577	DL/HL	-0.45	PPL/SL
4	0.606	HD/SL	0.408	IOW/HL	-0.405	HL/SL
5	0.602	PPL/SL	0.352	CPD/SL	-0.404	DAA/DVA

The BI and ML trees of *cyt b* and S7 have the same topology. *Garra imberba* from Jinshajiang, Red, Mekong River and *G. hainanensis* are clustered together, and *Garra nujiangensis* is the sister species of *G. imberba*. In general, phylogenetic relationships based on the phylogenetic trees (Figure 3) of mtDNA exhibit a marked pattern based on geographical distribution, which is different from the result of morphological analyses (Figure 2). *Garra hainanensis* has a closer relationship with *G. imberba* from Jinshajiang and the main stream of Red River, and then clustered with *G. imberba* from Lixianjiang and Lancangjiang River materials. The results show a conflict in the modern river systems; Lixianjiang River is the biggest tributary of Red River, but samples from Lixianjiang have a closer relationship with Lancangjiang River.

*G. nujiangensis* from Nujiang River can also be easily identified (Figure 4) based on the phylogenetic trees based on the S7, and a mix of samples from Jinshajiang, Red, Lixianjiang, Lancangjiang River and Hainan Island.

## DISCUSSION

### Morphological discrimination between species

Morphological characteristics are still the most important evidence for defining a new species or subspecies in cyprinid fish. Such analyses usually include two kinds of data: meristic and metric. A difference in meristic characteristics usually can be used as evidence for defining a new species; metric data usually is used to describe a new species or subspecies based on significant difference (Chu & Cui, 1987; Chu & Chen, 1989).

Table 2 shows the details of the morphological characters of these five species. Most of the meristic data do not have significant difference, except for the circumpeduncle scales: *G. imberba* usually has 16 or on rare occasions 18 (1 in 90) circumpeduncle scales; most of *G. nujiangensis* has 12, and only 1 sample has 14 circumpeduncle scales. Number of circumpeduncle scales was treated as very important evidence in defining species, such as *G. qiaojiensis* and *G. orientalis* (12 vs. 16)

Table 4 The pairwise *p* distances of *cyt b* computed by MEGA

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
<i>Garra imberba</i> YZ1																						
<i>Garra imberba</i> YZ2	0.000																					
<i>Garra imberba</i> RR1	0.001	0.001																				
<i>Garra imberba</i> RR2	0.001	0.001	0.002																			
<i>Garra imberba</i> LX1	0.025	0.025	0.026	0.026																		
<i>Garra imberba</i> LX2	0.028	0.028	0.029	0.029	0.003																	
<i>Garra imberba</i> MK1	0.028	0.028	0.029	0.029	0.017	0.019																
<i>Garra imberba</i> MK2	0.029	0.029	0.030	0.030	0.017	0.019	0.001															
<i>Garra hainanensis</i> CH1	0.008	0.008	0.009	0.009	0.028	0.031	0.032	0.033														
<i>Garra hainanensis</i> CH2	0.008	0.008	0.009	0.009	0.028	0.031	0.032	0.033	0.000													
<i>Garra nuijiangensis</i> SW1	0.095	0.095	0.096	0.096	0.091	0.092	0.095	0.094	0.097	0.097												
<i>Garra nuijiangensis</i> SW2	0.095	0.095	0.096	0.096	0.091	0.092	0.095	0.094	0.097	0.097	0.000											
<i>Garra micropulvinus</i>	0.104	0.104	0.105	0.105	0.096	0.099	0.103	0.102	0.110	0.110	0.106	0.106										
<i>Garra findolabium</i>	0.111	0.111	0.111	0.111	0.106	0.106	0.106	0.105	0.111	0.111	0.096	0.096	0.116									
<i>Garra cryptonemus</i>	0.128	0.128	0.129	0.129	0.128	0.128	0.130	0.129	0.129	0.129	0.129	0.129	0.128	0.143								
<i>Garra dulongensis</i>	0.139	0.139	0.139	0.139	0.132	0.134	0.132	0.131	0.139	0.139	0.136	0.136	0.141	0.129	0.148							
<i>Garra microfrontis</i>	0.128	0.128	0.127	0.129	0.121	0.124	0.123	0.122	0.126	0.126	0.127	0.127	0.123	0.125	0.142	0.105						
<i>Garra caudofasciatus</i>	0.123	0.123	0.124	0.124	0.120	0.121	0.127	0.126	0.121	0.121	0.119	0.119	0.118	0.113	0.150	0.138	0.140					
<i>Garra tengchongensis</i>	0.151	0.151	0.152	0.152	0.140	0.141	0.142	0.141	0.148	0.148	0.132	0.132	0.139	0.132	0.143	0.104	0.110	0.142				
<i>Garra orientalis</i>	0.124	0.124	0.125	0.125	0.118	0.120	0.119	0.118	0.125	0.125	0.125	0.125	0.118	0.128	0.139	0.105	0.058	0.143	0.105			
<i>Garra salweenica</i>	0.134	0.134	0.135	0.135	0.129	0.132	0.130	0.129	0.134	0.134	0.132	0.132	0.131	0.127	0.138	0.100	0.059	0.141	0.111	0.063		
<i>Garra qiaojiensis</i>	0.141	0.141	0.140	0.142	0.138	0.139	0.140	0.139	0.143	0.143	0.135	0.135	0.132	0.125	0.144	0.110	0.089	0.136	0.118	0.090	0.096	
<i>Garra fasciacauda</i>	0.126	0.126	0.126	0.127	0.120	0.123	0.125	0.125	0.126	0.126	0.126	0.126	0.130	0.127	0.145	0.131	0.127	0.144	0.130	0.121	0.127	0.132

Table 5 The pairwise *p* distances of s7 computed by MEGA

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
<i>Garra imberba</i> YZ1																						
<i>Garra imberba</i> YZ2	0.005																					
<i>Garra imberba</i> RR1	0.002	0.006																				
<i>Garra imberba</i> RR2	0.002	0.006	0.000																			
<i>Garra imberba</i> LX1	0.002	0.003	0.003	0.003																		
<i>Garra imberba</i> LX2	0.005	0.000	0.006	0.006	0.003																	
<i>Garra imberba</i> MK1	0.002	0.003	0.003	0.003	0.000	0.003																
<i>Garra imberba</i> MK2	0.002	0.003	0.003	0.003	0.000	0.003	0.000															
<i>Garra hainanensis</i> CH1	0.002	0.003	0.003	0.003	0.000	0.003	0.000	0.000														
<i>Garra hainanensis</i> CH2	0.002	0.003	0.003	0.003	0.000	0.003	0.000	0.000	0.000													
<i>Garra nuijiangensis</i> SW1	0.015	0.015	0.014	0.014	0.014	0.015	0.014	0.014	0.014	0.014												
<i>Garra nuijiangensis</i> SW2	0.015	0.015	0.014	0.014	0.014	0.015	0.014	0.014	0.014	0.014	0.000											
<i>Garra micropulvinus</i>	0.041	0.043	0.040	0.040	0.040	0.043	0.040	0.040	0.040	0.040	0.040	0.040										
<i>Garra findolabium</i>	0.038	0.040	0.037	0.037	0.037	0.040	0.037	0.037	0.037	0.037	0.037	0.037	0.044									
<i>Garra cryptonemus</i>	0.060	0.061	0.058	0.058	0.058	0.061	0.058	0.058	0.058	0.058	0.060	0.060	0.070	0.064								
<i>Garra dulongensis</i>	0.126	0.126	0.124	0.124	0.124	0.126	0.124	0.124	0.124	0.124	0.121	0.121	0.119	0.124	0.141							
<i>Garra mirofrontis</i>	0.113	0.115	0.112	0.112	0.112	0.115	0.112	0.112	0.112	0.112	0.112	0.112	0.112	0.113	0.135	0.072						
<i>Garra caudofasciatus</i>	0.051	0.052	0.049	0.049	0.049	0.052	0.049	0.049	0.049	0.049	0.046	0.046	0.055	0.052	0.070	0.139	0.129					
<i>Garra tengchongensis</i>	0.142	0.142	0.141	0.141	0.141	0.142	0.141	0.141	0.141	0.141	0.138	0.138	0.136	0.139	0.156	0.060	0.080	0.155				
<i>Garra orientalis</i>	0.118	0.119	0.116	0.116	0.116	0.119	0.116	0.116	0.116	0.116	0.116	0.116	0.116	0.118	0.139	0.077	0.014	0.132	0.087			
<i>Garra salweenica</i>	0.110	0.112	0.109	0.109	0.109	0.112	0.109	0.109	0.109	0.109	0.110	0.110	0.109	0.110	0.132	0.072	0.040	0.123	0.080	0.044		
<i>Garra qiaojiaensis</i>	0.123	0.124	0.121	0.121	0.121	0.124	0.121	0.121	0.121	0.121	0.121	0.121	0.121	0.123	0.142	0.078	0.052	0.136	0.084	0.060	0.057	
<i>Garra fasciacanda</i>	0.156	0.156	0.155	0.155	0.155	0.156	0.155	0.155	0.155	0.155	0.155	0.155	0.162	0.155	0.172	0.173	0.165	0.164	0.185	0.165	0.176	0.173

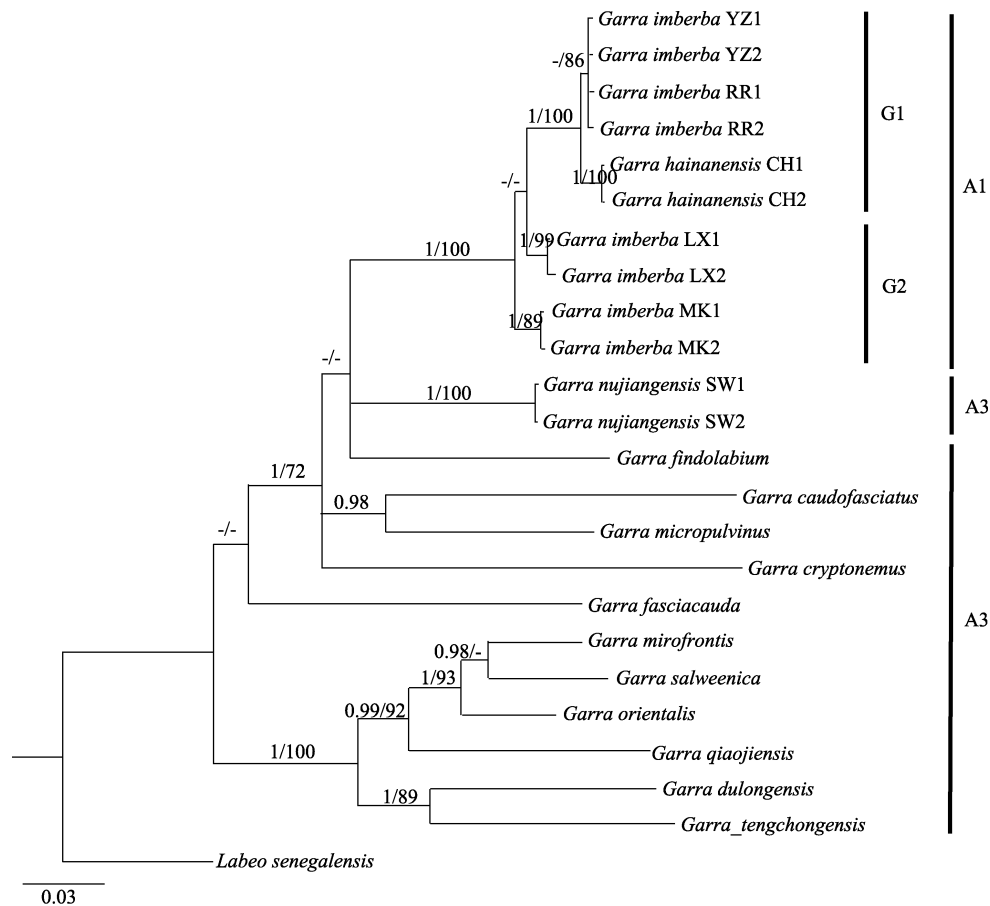
**Table 6 Overall mean p distance within and between clades computed by MEGA**

Sequence	Clade	Mean distance (%)	SE (%)
cyt b	A1	1.9	0.3
	A2	0	0
	A3	12.3	0.5
	A1 Vs. A2	9.5	0.8
	A1 Vs. A3	12.7	0.6
	A2 Vs. A3	12.4	0.6
S7	A1	0.2	0.1
	A2	0	0
	A3	11.2	0.7
	A1 Vs. A2	1.4	0.4
	A1 Vs. A3	9.7	0.7
	A2 Vs. A3	9.6	0.7

(Chu & Cui, 1987). *Garra nuijiangensis* can be distinguished from all the other species by having 12 circumpeduncle scales (vs. 16) which implies it is a valid species and distinct from *G. imberba*.

The number of branched dorsal fin rays is thought to be a stable characteristic and efficient in defining new species (Chu & Cui, 1987). In this study, we found it is actually not as stable as the circumpeduncle scales. For example, in *G. imberba*, among the 90 examined materials 2 samples have 10 branched dorsal fin rays, 10 have 8, and 72 have 9. The number of lateral line scales usually has a changing range and was treated as a general characteristic in defining species (Chu & Cui, 1987). Meristic data in Table 2 show that these two characteristics have little but not significant difference among *G. imberba*, *G. yiliangensis* and *G. hainanensis*, so it is not reasonable to treat them as distinct species based solely on the above characteristics.

In the PCA analysis, distinction among these five species is not very clear. From Figure 2, it can be seen that *G. yiliangensis* has the most significantly different from the other species, but still has 1 material mixed within it. Component 1 mainly stands for the CPD/CPL, which means most *G. yiliangensis* have a slender body, but the metric character is not reliable in defining species

Figure 3 BI tree constructed using MrBayes based on *cyt b* gene

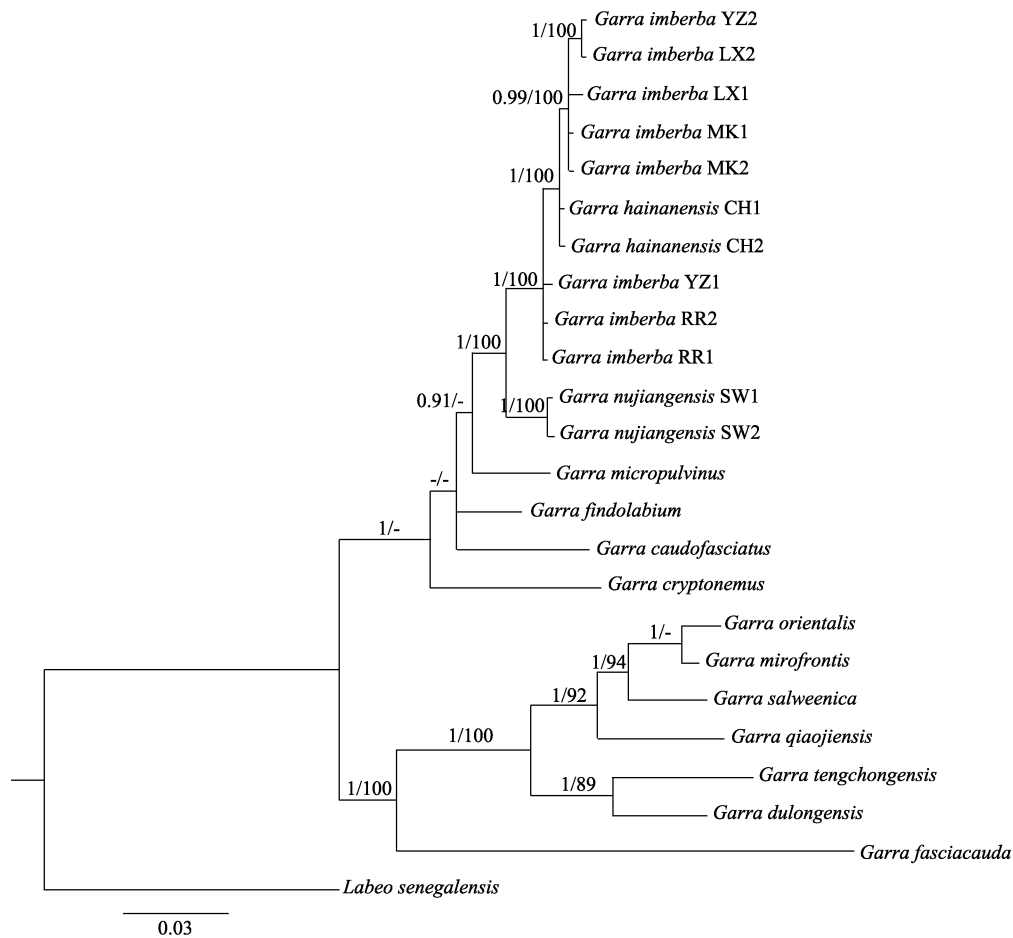


Figure 4 BI tree constructed using MrBayes based on the S7

especially in genus *Garra* (Chu & Cui, 1987). The morphological characteristic is closely related with the environment, for example eyes will degenerate if the fish live in dark caves for a long time. *Garra yiliangensis* has a slender body especially for the caudal peduncle. From an ecological view, it is a characteristic adopted for fast flowing waters. But this difference is not significant, which implies that *G. yiliangensis* from Nanpanjiang River only recently became isolated from adjacent rivers, so it should have a close relationship with *G. imberba* from Jinshajiang and Red River.

*Garra alticorpora* can only be distinguished from other related species by the fact that its body depth is longer than its head length. There is no difference in meristic characteristics with *G. imberba* and mixed with the others in PCA analysis. The only one holotype and one paratype were collected in Pingbian, Yunnan, which belongs to the Red River Drainage system. In the following surveys, however, only *G. imberba* were caught, so the two specimens of *G.*

*alticorpora* may be just a special case or gravid females and should be treated as a synonym of *G. imberba* (Zhou et al, 2005).

From traditional morphological characteristics analyses, only *G. nujiangensis* can be distinguished from other species. *Garra imberba*, *G. yiliangensis*, *G. hainanensis* and *G. alticorpora* are relatively indifferent from one another, and should be assigned to the same species. Because of the geographical isolation, *G. yiliangensis* and *G. hainanensis* should be treated as different geographical populations of *G. imberba*.

### Molecular phylogenetic relationship

It is widely accepted that Jinshajiang, Nanpanjiang and Red River have a close relationship; indeed, Lancangjiang, Nujiang and Irrawaddy River have a close relationship (Chu, 1986). Based on geological studies, sea-level changes during the Pleistocene may have reached the maximum lows, which means the

Red River might have been linked together with rivers on Hainan Island (Hanebuth *et al.*, 2000; Voris, 2000). Therefore, *G. hainanensis* and *G. imberba* from Red and Jinshajiang River possibly have the same ancestor, and split only after the Pleistocene era, which may explain why *G. hainanensis* has a closer relationship with *G. imberba* from Red and Jinshajiang River, and why they were clustered in a lineage of G1. The close relationship coincides with the geographical process of the three rivers.

The Mekong River has the highest fish diversity in Southeast Asia and its fish fauna is also different from the Red, Jinshajiang and Nanpanjiang Rivers (Froese & Pauly, 2012; Yap, 2002; Zakaria-Ismail, 1994). Lixianjiang (Song Da in Vietnam) is the biggest tributary of the Red River, which originated from the Wuliang Mountain, Yunnan, China and flows into the Red River at Viet Tri, Vietnam, but the mtDNA analysis shows that, specimens from Lixianjiang River have a closer relationship with Lancangjiang than the Red River. Zhou *et al.* (2010) compared the morphometrics of *Vanmanenia tetraloba* from Lancangjiang, Lixianjiang and main stream of Red River. Their results show that specimens from different rivers actually represented different species. *Garra imberba* is not a fish that migrates long distances, and so a gene exchange between the main stream of Red and Lixianjiang would be very difficult given the distance. Therefore, *G. imberba* from Lixianjiang and the main stream of the Red River would not have exchanged genes for a long time, and they were not clustered in the same clade. Clade A1 can be divided into two lineages, G1 and G2, which coincide with the geographical process and the fish fauna of the rivers but conflict with the morphological classification of *G. imberba* and related species. Furthermore, the mean *p*-distance within the clade A1 is 1.9%, and the biggest *p*-distance within clade A1 is 3.2%, which is between *G. hainanensis* and *G. imberba* from Lancangjiang River. This value is smaller than the minimum value within the genus *Garra* (Yang *et al.*, 2012). With respect to the evolutionary history and phylogenetic relationship of the samples from different rivers, it is better to treat them as the same species.

The 1st intro of S7 ribosomal protein gene was thought to be an efficient marker for the phylogenetic analyses of subfamily level (He *et al.*, 2008). It has a

faster evolution rate than the coding nuclear gene but a slower rate than mtDNA, so we used it here to explore the relationship at a species level. Based on the NJ tree of S7 data, *G. nujiangensis* from Nujiang River and *G. micropulvinus* can be easily identified (Figure 4), which implies *G. nujiangensis* is a valid species and S7 gene is an efficient means for exploring phylogenetic relationships within genus *Garra*. *Garra imberba* and *G. hainanensis* were mixed together and the mean *p*-distance within this clade is 0.2%. It is obvious that *G. imberba* and *G. hainanensis* are the same species and distinct from *G. nujiangensis*.

## CONCLUSION

For the morphological analysis, a stable meristic characteristic can be treated as an important proof when defining a new species, such as circumpeduncle scales, but metric characteristics should be considered carefully, especially in exploring relationships between species. Molecular phylogenetic analysis is an efficient method in estimating the phylogenetic relationships between different species or geographical groups. To make a reasonable taxonomy, the morphological differences, phylogenetic relationship and the biogeographic processes should all be considered.

In this study, both morphologic and molecular analyses testified that *G. nujiangensis* is a valid species and distinct from *G. imberba*. Samples from Jinshajiang, Red, Lixianjiang, Nanpanjiang, Lancangjiang River and Hainan Island have the same ancestor, which was isolated because of the formation of modern river systems. Different environments usually lead to different morphological characteristics, but the differences between these materials is not enough to define new species. Based on the morphological and molecular analyses, the samples from Jinshajiang, Nanpanjiang, Red, Lancangjiang River and Hainan Island have a close phylogenetic relationship and should be treated as the same species, and because of the geographical isolation, *G. yiliangensis* and *G. hainanensis* should be treated as different geographical populations of *G. imberba*.

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## Materials examined

### *Garra imberba*

Lancangjiang River: KIZ200800826-829, 3, 97.8–182.1 mm SL, Zhaji River, Jingfu Xiang, Jingdong, Yunnan; KIZ2002008504-8512, 9, 60.5–103.3 mm SL, Mengga River, Jinggu, Yunnan; KIZ2008000087-89, 2, 83.1–128.6 mm SL, Menggen county, Lancang, Yunnan; KIZ2008008269, 1, 97.6 mm SL, Puer, Yunnan.

Main stream of Red River: KIZ1964000024-25, 2, 14.7–16.2 mm SL, Yuanjiang, Yunnan; KIZ2007002528, 1, 19.4 mm SL, Hekou, Yunnan; KIZ2008000922, KIZ2008000927, KIZ2008000929, KIZ2008000932, KIZ2008000936, KIZ2008000938-940, 8, 15.1–20.7 mm SL, Namoguo, Wenshan, Yunnan; KIZ2010010001, 1, 102.9 mm SL, Xinping, Yunnan; KIZ2010010002, 1, 71.6 mm SL, Simao, Yunnan.

Jinshajiang River: KIZ1982001210-1224, 15, 47.4–118.85 mm SL, Yanjin, Yunnan; KIZ2004011252-253, 2, 69.8–195.0 mm SL, Yongsheng, Lijiang, Yunnan; KIZ2008006836, KIZ2010001891, 2, 101.5–136.33 mm SL, Huize, Yunnan; KIZ041110093-99, 6, 91.2–150.7 mm SL, Yongren, Yunnan; KIZ1977000732-734, KIZ1977-000737-738, 5, 51.1–73.1 mm SL, Heqing, Yunnan; KIZ2008006837-40, 4, 108.5–159.3 mm SL, Huize, Yunnan.

Lixianjiang River: KIZ2000001439-1454, 16, 64.5–163.1 mm SL, Jinping, Yunnan; KIZ2007002903-2906, 4, 68.3–107.2 mm SL, Jiangcheng, Yunnan; KIZ2008002405-2416, 12, 102.0–130.6 mm SL, Simao, Yunnan; KIZ2004001193, 1, 82.0 mm SL, Puer, Yunnan; KIZ2009002070, 1, 53.8 mm SL, Lvchun, Yunnan.

### *Garra yiliangensis*

KIZ1960000569, KIZ1963000385, KIZ197700093-3-934, 4, 146.1–187.0 mm SL, Yiliang, Yunnan; KIZ1977000954-958, 5, 166.3–252.5 mm SL, Luoping, Yunnan.

### *Garra hainanensis*

KIZ2008003938, KIZ2008003947, KIZ2008003967, KIZ2008003973-3974, KIZ2008003979, KIZ2008003990, KIZ2008003995, KIZ2008004006, KIZ2008004018, KIZ2008004030, KIZ2008004039, 12, 93.6–117.7 mm SL, Ledong, Hainan.

### *Garra nujiangensis*

KIZ2003007967 (Holotype), KIZ2003000279 (Paratype), KIZ2003000272, KIZ2003000278, KIZ2003000281-282, KIZ2003000288-289, KIZ2003000291, KIZ2003000294, 10, 82.5–186.7 mm SL, Zhenkang, Yunnan; KIZ2005005140-145, 6, 117.8–173.0 mm SL, Cangyuan, Yunnan;

### *Garra alticorpora*

KIZ1985001344 (Holotype), KIZ1985001345 (Paratype), 2, 164.6–165.6 mm SL, Pingbian, Yunnan.

### *Garra micropulvinus*

KIZ2008005732, 1, 83.4 mm SL, Wenshan, Yunnan.

### *Garra findolabium*

KIZ2011002800, 1, 36.9 mm SL, Jinping, Yunnan.

### *Garra cryptonemus*

KIZ5171, 1, 13.5 mm SL, Liuku, Yunnan.

### *Garra caudofasciatus*

KIZ2007002789, 1, 76.1 mm SL, Jiangcheng, Yunnan.

### *Garra fasciacauda*

KIZ2004014928, 1, 56.3 mm SL, Puer, Yunnan.

### *Garra tengchongensis*

KIZ2006004460, 1, 53.3 mm SL, Tengchong, Yunnan.

### *Garra dulongensis*

KIZ2004000816, 1, 98.5 mm SL, Dulongjiang, Yunnan.

### *Garra qiaojiensis*

KIZ2006004422, 1, 112.1 mm SL, Yingjiang, Yunnan.

### *Garra salweenica*

KIZ2006003543, 1, 74.6 mm SL, Dehong, Yunnan.

### *Garra orientalis*

KIZ2005000086, 1, 138.9 mm SL, Longlin, Yunnan.

### *Garra mirofrontis*

KIZ2005002392, 1, 88.6 mm SL, Yunxian, Yunnan.

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## Descriptions of two new record species of *Scutellonema* (Nematoda: Tylenchida) from China

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**Abstract:** From 2011 to 2012, we collected *Scutellonema commune* from the rhizosphere soil of coconut (*cocos nucifera* L.) and *Scutellonema magniphasma* from the rhizosphere soil of *Rosa chinensis* in Shenyang, China. *S. commune* was characterized by rounded scutella, which was varied from two annules anterior to the anus and five annules posterior. The lateral field was not areolated at the level of scutella. *S. magniphasma* was characterized by varying scutellum, about 7.0  $\mu\text{m}$  in diameter, in position from four annules posterior to four annules anterior to the anus, and areolated at the level of the scutellum.

**Keywords:** Hoplolaimidae; new record species; *Scutellonema commune*; *Scutellonema magniphasma*

The genus *Scutellonema* (Sivakumar, 1981) was established by Andrassy in 1958, and is characterized by scutella (enlarged phasmids) both opposing and being near to the anus. Presently, the number of species in this genus has reached 31, and all were either ectoparasites or partially endoparasites of plants.

*Scutellonema commune* (Germani, 1985) was first described by van den Berg and Heyns in 1973, belonging to Nemata, Tylenchida, Hoplolaiminae. Its body curves notably toward the ventral side, forming complete circle under heat. The scutellum is rounded, but not areolated at level of the scutella.

*S. magniphasma* (Germani, 1985) was described by Sher in 1963 but earlier collected in 1962 by Martin from the University of California. Its body is spirally curved or C-shaped, with a large scutellum (7.0  $\mu\text{m}$  in diameter).

In this study, we report two new species records, *S. commune* and *S. magniphasma*. These two new recorded species were collected in a survey of the ornamental plant-parasitic nematodes in Shengyang, located in the northeast of China, 2011.

## MATERIALS AND METHODS

Specimens of *S. commune* were obtained from the rhizosphere soil of coconut (*Cocos nucifera* L.) in a flower greenhouse located in Shenyang, Liaoning Province, China. Specimens of *S. magniphasma* were

meanwhile obtained from the rhizosphere soil of *Rosa chinensis* on the campus of Shenyang Agricultural University. After sieving-centrifugal flotation, nematodes were euthanized by gentle heat. Specimens were then fixed and preserved in FA 4:1 and were mounted in anhydrous glycerol on permanent slides (Seinhorst, 1959) to allow for measuring and photography. Specimens were observed under light microscope equipped with Motic BA400 and measured by Motic Images Advanced 3.2.

## RESULTS

*S. commune* van den Berg and Heyns, 1973 (Figure 1, 2).

### Measurements

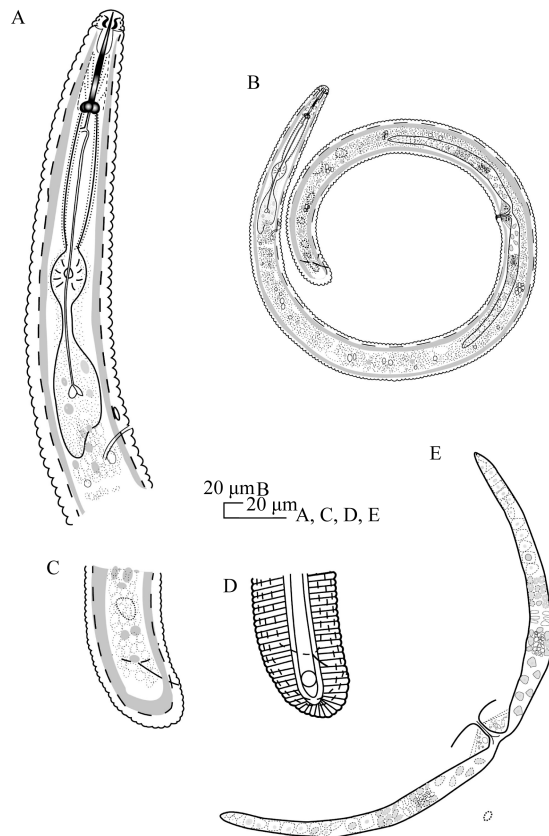
Female ( $n=10$ ):  $L=766$  (700–860)  $\mu\text{m}$ ;  $a=24$  (22–30);  $b=6.8$  (6.0–7.5);  $b'=5.6$  (4.7–5.8);  $c=46$  (41–62);  $c'=0.7$  (0.6–0.9);  $V=56$  (53–65);  $st=30.3$  (27–32)  $\mu\text{m}$ ;  $o=22$  (18–23); scutellum diameter=3.9 (2–6)  $\mu\text{m}$ .

$L$ : body length;  $a$ : body length/greatest body width;  $b$ : body length/distance from anterior end to junction of oesophagus and intestine;  $b'$ : body length/distance from anterior end to oesophagus end;  $c$ : body length/tail length;

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 Figure 1 Illustrations of *S. commune*

A: Anterior region; B: Female entire; C, D: Tail. E: Senital system;


 Figure 2 *S. commune* under light microscope

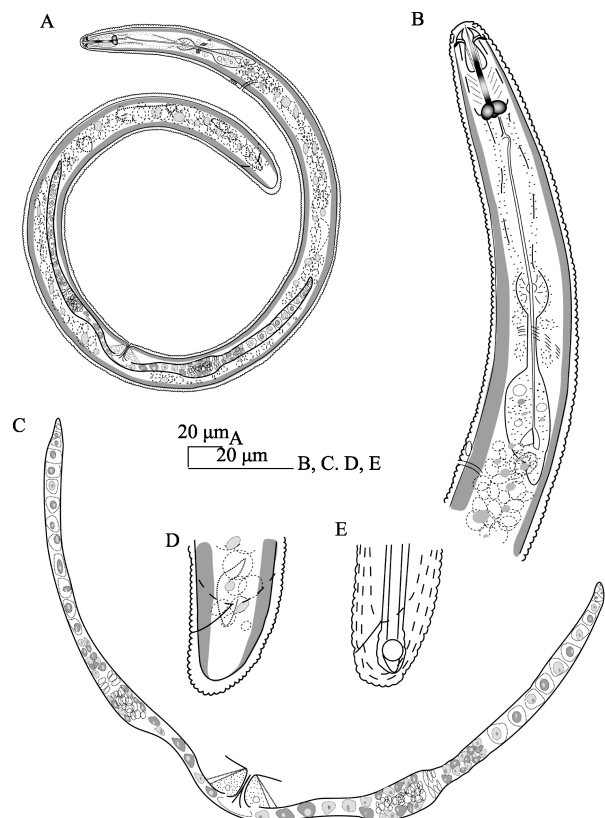
 A: Female individual; B: Head; C: Metacarpus; D: Vulva region; E: Esophageal gland; F, G: tail; Scale bars=20  $\mu$ m.

c': tail length/anus body width; V: distance from vulva to anterior end $\times$ 100/body length; st: stylet length; o: dorsal gland orifice to stylet/ stylet length

## Description

Female: Body curved notably toward ventral side, forming complete circle upon heating. Annules were 1.3  $\mu$ m wide at midbody. Lip region was hemispherical with 3–4 annules and was separated from body by a slight constriction, without longitudinal striation. Spear was well-developed, oval basal knobs with irregular anterior surface (Figure 1C, Figure 2B). Opening of dorsal oesophageal gland was 4–10  $\mu$ m from basal knobs. Excretory pore was leveled with oesophageal gland lobe and was 127  $\mu$ m (108–149) from anterior extremity. Hemizonid was 1–3 annules anterior to excretory pore. Vulva glands were large and elongated. Lateral field was 6–8  $\mu$ m wide, with four incisures, and was not areolated at the level of scutella. Scutellum was rounded (Figure 1E, F; Figure 2F), varying from two annules anterior to and five annules posterior to anus. Tail was rounded and was 13.7  $\mu$ m (11–22) in length.

Male: Unknown.


 Figure 3 Illustrations of *S. magniphasma*

A: Female individual; B: Anterior region; C: Senital system; D, E: Tail.

*Scutellonema magniphasma* Sher, 1963 (Figure 3, 4).

### Measurements

Female ( $n=10$ ):  $L=891(820-980)$   $\mu\text{m}$ ;  $a=27(24-30)$ ;  $b=7.3(7-8)$ ;  $b'=5.2(4.7-5.8)$ ;  $c=53(48-75)$ ;  $c'=0.57(0.5-0.8)$ ;  $V=56(55-61)$ ;  $st=31(29-35)$   $\mu\text{m}$ ;  $o=13(12-17)$ ; scutellum diameter= $6.5(5-7)$   $\mu\text{m}$

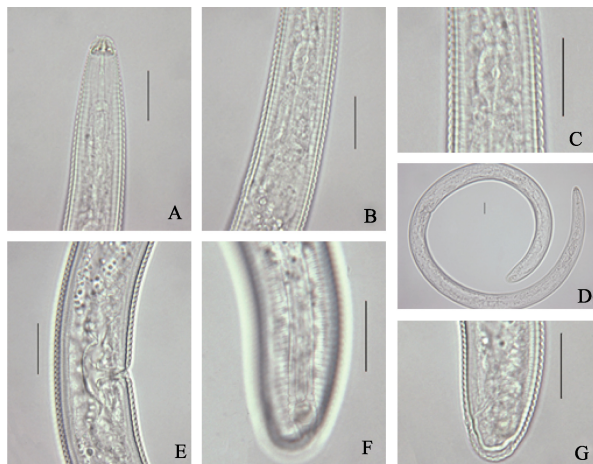


Figure 4 *S. magniphasma* under light microscope

A: Head; B: Esophageal gland; C: Metacarpus; D: Female entire; E: Vulva region; F, G: Tail; Scale bars=20  $\mu\text{m}$ .

### Description

Female: Body spirally curved or C-shaped when killed by heat. Annules were 2.3  $\mu\text{m}$  wide at midbody. Lip region was hemispherical and slightly offset, with 3–5 annules, but some variants were conoid and deeply offset. Basal lip annule was with 20–26 longitudinal striations. Spear was well-developed, oval basal knobs

with flattened surface (Figure 3C; Figure 4A). Opening of the dorsal oesophageal gland was 6–10  $\mu\text{m}$  from the basal knobs. Excretory pore was 133 (118–152)  $\mu\text{m}$  from the anterior extremity and was at the level of oesophageal gland lobe. Hemizonid was 0–4 annules anterior to excretory pore. Vulva glands were large and elongated. Lateral field was with four incisures and areolated at scutellum. Scutellum was varying from four annules posterior to four annules anterior to anus (Figure 3E; Figure 4F). Tail was tapering and rounded.

Male: Unknown.

### DISCUSSION

The morphological data and characteristics of *S. commune* were consistent with previous records except a shorter spear (30  $\mu\text{m}$  vs. 32  $\mu\text{m}$ ) on average (van den Berg & Heyns, 1973). *S. commune* is similar to *S. africanum* Smit, 1971 in the absence of areolation at the level of the scutellum. However, *S. commune* is distinguished by its slightly set-off head region, larger body sizes (0.7–0.9 mm vs. 0.51–0.81 mm) and spear (27–36  $\mu\text{m}$  vs. 20–28  $\mu\text{m}$ ).

The morphological data and characteristics of *S. magniphasma* were consistent with previous records, save for the observed longer body (900  $\mu\text{m}$  vs. 820  $\mu\text{m}$ ) on average (Sher, 1963). *S. magniphasma* is similar to *S. unum* Sher, 1964 in areolation at the level of scutellum and ten or more longitudinal striae on the basal lip annule. That said, it could be differentiated from *S. unum* by the comparatively larger scutella (5–7  $\mu\text{m}$  vs. 4  $\mu\text{m}$ ).

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## Effects of temperature acclimation on body mass and energy budget in the Chinese bulbul *Pycnonotus sinensis*

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**Abstract:** Chinese bulbuls (*Pycnonotus sinensis*) are small passerine birds that inhabit areas of central, southern and eastern China. Previous observations suggest that free-living individuals of this species may change their food intake in response to seasonal changes in ambient temperature. In the present study, we randomly assigned Chinese bulbuls to either a 30 °C or 10 °C group, and measured their body mass (BM), body temperature, gross energy intake (GEI), digestible energy intake (DEI), and the length and mass of their digestive tracts over 28 days of acclimation at these temperatures. As predicted, birds in the 30 °C group had lower body mass, GEI and DEI relative to those in the 10 °C group. The length and mass of the digestive tract was also lower in the 30 °C group and trends in these parameters were positively correlated with BM, GEI and DEI. These results suggest that Chinese bulbuls reduced their absolute energy demands at relatively high temperatures by decreasing their body mass, GEI and DEI, and digestive tract size.

**Keywords:** Body mass; Energy budget; *Pycnonotus sinensis*; Temperature acclimation

Thermogenesis and adjustment of energy intake are important for the survival of winter—particularly active birds in their natural environment (O'Connor, 1995, Yuni & Rose, 2005). The daily energy expenditure of many birds is usually higher in winter (Bryant et al, 1985; O'Connor, 1995; Stokkan et al, 1986) and this change may be triggered by environmental factors such as photoperiod, ambient temperature, and diet quality and/or quantity (Starck, 1996; 1999; Boon et al, 2000; Klaassen et al, 2004). Temperature is an important environmental *zeitgeber* for seasonal acclimatization in birds (McKechnie et al, 2007; Swanson, 2001; Tieleman et al, 2003; Vézina et al, 2006). Low-temperature stress during winter in temperate zones may induce an increase in energy expenditure during a season of generally decreased food availability (Krams et al, 2010). Many birds have a variety of strategies to cope with low temperature stress, such as increasing body mass, food intake and thermogenesis (Bednekoff et al, 1994; Goymann et al, 2006; Krams et al, 2010; Webster & Weathers, 2000; Williams & Tieleman, 2000; Swanson, 2010). The body mass of a bird is the sum of its energy

intake and energy expenditure. In a bird maintaining constant body mass, time-averaged energy intake equals time-averaged energy use (Hammond & Diamond, 1997). This balance depends on the interplay between the intake and digestive processing of matter and energy, and their allocation among diverse functions, including thermoregulation, growth and reproduction (Caviedes-Vidal et al, 2007). Accordingly, morphological changes in the digestive tract provide a useful indication of energy expenditure (Williams & Tieleman, 2000; Karasov et al, 2011).

Phenotypic flexibility is a response to environmental conditions varying predictably, or of more stochastic fluctuations in the environment (Piersma & Drent, 2003; Starck & Rahmaan, 2003). Such responses to variable conditions ought to be reversible and repeatable

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(McWilliams & Karasov, 2001; Starck & Rahmaan, 2003). Several authors suggest that the avian digestive tract is a suitable model for the study of phenotypic plasticity because its morphology is positively correlated with food composition (frugivorous and nectar-feeding birds have smaller and shorter digestive tracts than granivorous and insectivorous species), and depends not only on resorption and assimilation processes, but also on fine adjustment of its morphology, such as epithelial resorptive surface dimensions, volume and transport efficiency (DeGoliér *et al.*, 1999; Goymann *et al.*, 2006; Karasov *et al.*, 2004; Lavin *et al.*, 2008; Starck & Rahmaan, 2003; Webster & Weathers, 2000).

Chinese bulbuls (*Pycnonotus sinensis*) are small passerines that are distributed over vast areas of central, southern and eastern China (MacKinnon & Phillipps, 2000). They have been reported to have high body temperature ( $T_b$ ), high upper critical temperature ( $T_{uc}$ ), low BMR, a relatively wide thermal neutral zone (TNZ) (Zhang *et al.*, 2006), and they increase their body mass and BMR in response to colder temperatures (Zheng *et al.*, 2008b). There is evidence that this species enhances its thermogenic capacity by increasing organ mass and respiratory enzyme activity (Zhang *et al.*, 2008; Zheng *et al.*, 2010). For this study, we selected Chinese bulbuls as a study species because they are resident in Zhejiang Province where we are based, and global warming appears to have allowed this species to colonize northeastern and northwestern China (Li *et al.*, 2006; Song, 2006), and previous studies (Ni *et al.*, 2010; Peng *et al.*, 2010; Zhang *et al.*, 2006, 2008; Zheng *et al.*, 2008a, 2010; Zhou *et al.*, 2010) provide critical background information required for our research. In this report, we investigated changes in body mass, energy budget and digestive tract morphology under two different metabolic loads, or energy demands, elicited by ambient temperature. We hypothesized that phenotypic variation in body mass, energy budget and digestive organs may play important roles in the adaptation of Chinese bulbuls to changing environmental conditions. We predicted that Chinese bulbuls will decrease their body mass and energy intake in response to warm ambient temperatures similar to those typically experienced in summer. Furthermore, when individuals have low energy requirements we predict that the mass and length of their digestive tract will decrease accordingly.

## MATERIALS AND METHODS

In Wenzhou, the climate is warm-temperate with an average annual rainfall of 1 700 mm spread across all months, with slightly more precipitation during winter and spring. The mean annual temperature is 18 °C. Mean maximum daily temperature ranges from 39 °C in July to

8 °C in January, and mean minimum daily temperature from 28 °C in July to 3 °C in the same month. Mean winter and summer temperatures are 6 °C and 31 °C, respectively (Zheng *et al.*, 2008b).

## Animals

Chinese bulbuls were captured in mist nets in Wenzhou city during March 2011. Body mass to the nearest 0.1 g was determined immediately upon capture with a Sartorius balance (model BT25S). Birds were transported to the laboratory on the day of capture and caged outdoors for 1 or 2 d in 50×30×20 cm enclosures under natural photoperiods and temperatures of 13±1 °C before temperature acclimatization and measurements commenced. Food and water were supplied *ad libitum*. Birds were moved into individual cages for at least two weeks, after which 16 birds were randomly assigned to either the 30 °C or 10 °C group (eight birds in each group). Both groups were acclimated to these temperatures for 28 days. Body temperature was measured at 21:00 and 23:00 using a digital thermometer (Beijing Normal University Instruments Co.). The probe of the thermometer was inserted 3 cm into each bird's cloaca and a reading taken within 30 s.

## Energy budget

We regarded digestible energy intake as an index of total daily energy expenditure. During the 28 day experimental period, food was provided quantitatively and water was provided *ad libitum*. Food residues and feces were collected during the two days before temperature acclimation began (week 0) and once a week (every seventh day) thereafter throughout the four week experimental period. These residues were separated manually, then oven-dried at 60 °C until a constant mass was obtained. The caloric content of the residual food and feces were determined using a C200 oxygen bomb calorimeter (IKA Instrument, Germany). Gross energy intake (GEI), feces energy (FE), digestible energy intake (DEI), and digestibility of energy were calculated according to Grodzinski & Wunder (1975) and Ni *et al.* (2010):

$$\text{GEI (kJ/day)} = \text{dry food intake (g/day)} \times \text{caloric value of dry food (kJ/g)}$$

$$\text{FE (kJ/day)} = \text{dry mass of feces (g/day)} \times \text{caloric value of dry feces (kJ/g)}$$

$$\text{DEI (kJ/day)} = \text{GEI (kJ/day)} - \text{FE (kJ/day)}$$

$$\text{Digestibility (\%)} = \text{DEI (kJ/day)} / \text{GEI (kJ/day)} \times 100\%$$

## Measurements of organ masses

Following the observation period, all birds were killed humanely and their digestive tracts (gizzard, small intestine and rectum) were removed, measured (±1 mm) and weighed (±0.1 mg) at the end of the 28 day acclimation period. The gizzard, small intestine and

rectum were then rinsed with saline to remove all gut contents before being dried and reweighed. These organs were then dried to a constant mass over 2 d at 75 °C and reweighed to the nearest 0.1 mg (Liu & Li, 2006; Williams & Tieleman, 2000).

### Statistics

Data were analyzed using SPSS 12.0. All variables were tested for normality using the Kolmogorov-Smirnov test. Non-normally distributed data were transformed to natural logarithms. Repeated-measures analysis of variance (RM-ANOVA) was used to determine the significance of changes in body mass, body temperature, GEI, FE, DEI and digestibility over time. Least significant difference (LSD) *post hoc* tests were used to detect significant differences in the above parameters within the same group of birds between different days of temperature acclimation. The independent sample *t*-test was used to assess the significance of any differences in the body mass between 10 °C and 30 °C birds measured on the same day. Differences in the above variables between 10 °C and 30 °C birds, except for those in body mass, were evaluated using ANOVA or ANCOVA with body mass as a covariate where appropriate. In the case of digestibility, which was a percentage value, the arcsine-square-root transformation was performed prior to analysis to normalize the data. All results are expressed as mean±SEM, with  $P<0.05$  being considered statistically significant.

## RESULTS

There were no significant differences in body mass, body temperature, GEI, FE, DEI or digestibility between the 10 °C and 30 °C birds prior to the experiment.

### Body mass and body temperature

The body mass of the 30 °C birds decreased significantly throughout the course of the experiment (RM-ANOVA,  $F_{4,35}=2.718$ ,  $P<0.05$ , Figure 1A). Although this decrease in body mass was not significant after day 7 or day 14 (*post hoc*,  $P>0.05$ ), it was after day 21 (*post hoc*,  $P<0.05$ ) and by day 28 the body mass of the 30 °C group had decreased by 11.2% relative to that measured on day 0 (*post hoc*,  $P<0.05$ ). In contrast, the 10 °C birds maintained relatively constant body masses over the duration of the experiment (RM-ANOVA,  $F_{4,35}=0.420$ ,  $P>0.05$ ).

There was no significant difference in body mass between the 30 °C and 10 °C birds after day 7 ( $t_{14}=0.550$ ,  $P>0.05$ ), day 14 ( $t_{14}=0.196$ ,  $P>0.05$ ) and day 21 ( $t_{14}=0.2126$ ,  $P>0.05$ ), but there was after day 28 ( $t_{14}=2.371$ ,  $P<0.05$ ). At the end of experiment, the body mass of the 30 °C group was 7.5% lower than that of the 10 °C group (Figure 1A).

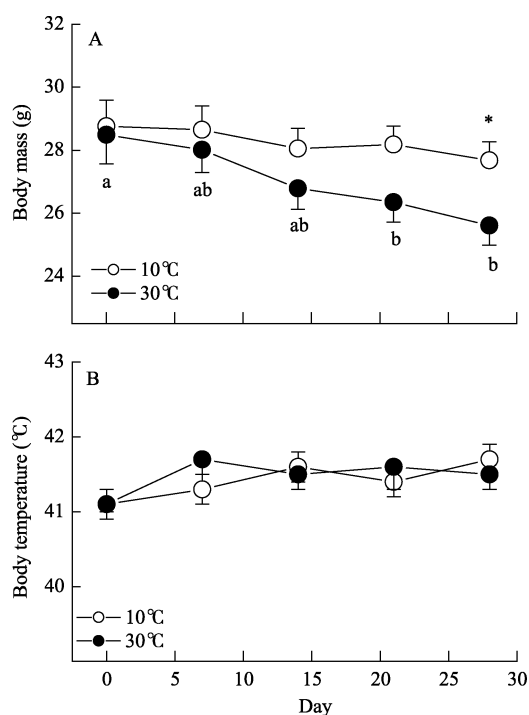


Figure 1 Trend in body mass and body temperature in Chinese bulbuls acclimated to either 30 °C or 10 °C

Data are presented as mean±SE; Different letters (a or b) indicate significant differences ( $P<0.05$ ) between measurements made on birds acclimated to 30 °C on different days; \*:  $P<0.05$ .

Neither the 10 °C nor the 30 °C group underwent significant changes in body temperature during the course of the experiment (RM-ANOVA, 10 °C,  $F_{4,35}=1.861$ ,  $P>0.05$ ; 30 °C,  $F_{4,35}=1.349$ ,  $P>0.05$ , Figure 1B), nor were any significant between-group differences in body temperature detected (day 7,  $F_{1,14}=2.679$ ,  $P>0.05$ ; day 14,  $F_{1,14}=0.009$ ,  $P>0.05$ ; day 21,  $F_{1,14}=0.260$ ,  $P>0.05$ ; day 28,  $F_{1,14}=0.531$ ,  $P>0.05$ ).

### Energy intake and digestibility

Birds in the 10 °C group did not undergo significant changes in FE (RM-ANOVA,  $F_{4,34}=2.239$ ,  $P>0.05$ , Figure 2B) or digestibility (RM-ANOVA,  $F_{4,34}=1.283$ ,  $P>0.05$ , Fig. 2D), but did undergo significant changes in GEI (RM-ANOVA,  $F_{4,34}=6.494$ ,  $P<0.01$ , Figure 2A) and DEI (RM-ANOVA,  $F_{4,34}=6.494$ ,  $P<0.001$ , Figure 2C). Although no significant change in these two parameters occurred during the first three weeks of the experiment, both had decreased significantly by day 28 (*post hoc*,  $P<0.05$ ).

Birds in the 30 °C group underwent significant changes in GEI, FE, DEI and digestibility (RM-ANOVA, GEI,  $F_{4,34}=24.508$ ,  $P<0.001$ , Fig. 2A; FE,  $F_{4,34}=15.741$ ,  $P<0.001$  Figure 2B; DEI  $F_{4,34}=22.236$ ,  $P<0.001$  Figure 2C; digestibility,  $F_{4,34}=3.438$ ,  $P<0.05$ ; Figure 2D). Significant changes in GEI, FE and DEI were evident by

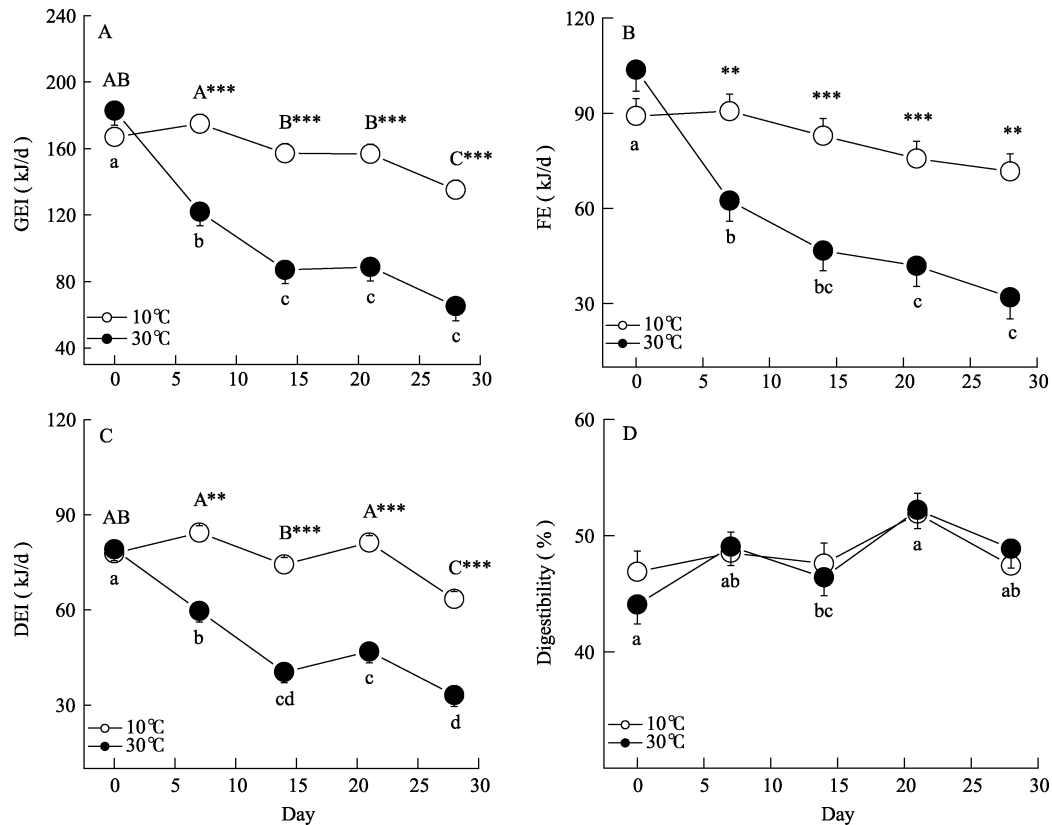


Figure 2 Trends in GEI, FE, DEI and digestibility in Chinese bulbuls acclimated to either 30 °C or 10 °C

Data are presented as mean±SEM; Different letters (a, b, c, or d for the 30 °C group; and A, B or C for the 10 °C group) indicate significant differences ( $P < 0.05$ ) in measurements made on birds in either the 30 °C or 10 °C group on different days; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$  indicate significant differences between the group of birds acclimated to 30°C and those acclimated to 10 °C.

day 7 (*post hoc*, GEI,  $P < 0.05$ ; FE,  $P < 0.05$ ; DEI,  $P < 0.05$ ) and continued over the subsequent three weeks. The GEI, FE and DEI of the 30 °C group were significantly lower than those of the 10 °C group on days 7, 14, 21 and 28 (ANCOVA, day 7, GEI,  $F_{1,13}=24.271$ ,  $P < 0.001$ ; FE,  $F_{1,13}=14.812$ ,  $P < 0.01$ ; DEI  $F_{1,13}=14.395$ ,  $P < 0.01$ ; day 28, GEI,  $F_{1,13}=46.130$ ,  $P < 0.001$ ; FE,  $F_{1,13}=18.252$ ,  $P < 0.01$ ; DEI  $F_{1,13}=96.832$ ,  $P < 0.001$ ). No significant difference in digestibility between the 10 °C and 30 °C groups was observed (ANCOVA, day 28,  $F_{1,13}=0.305$ ,  $P > 0.05$ ). Log GEI and DEI were positively correlated with log body mass at day 28 (GEI:  $r^2=0.372$ ,  $P < 0.01$ ; DEI:  $r^2=0.458$ ,  $P < 0.01$ , Figure 3).

### Digestive tract morphology

Although no significant changes in gizzard and rectum length (ANCOVA, gizzard,  $F_{1,13}=0.027$ ,  $P > 0.05$ ; rectum,  $F_{1,13}=1.108$ ,  $P > 0.05$ , Figure 4) were detected, the length of the total digestive tract and small intestine varied significantly over the course of the experiment (ANCOVA, total digestive tract,  $F_{1,13}=5.325$ ,  $P < 0.05$ ;

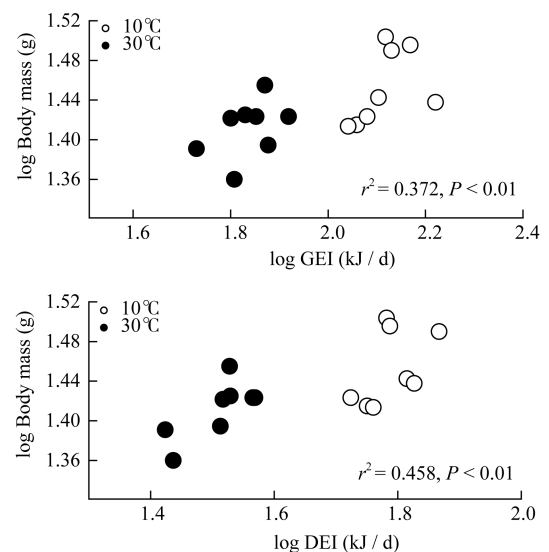


Figure 3 Least squares regression of GEI and DEI as dependent variable of body mass in Chinese bulbuls acclimated to 30 °C or 10 °C

small intestine,  $F_{1,13}=5.626$ ,  $P<0.05$ , Figure 4), becoming shorter in 30 °C birds than in 10 °C birds. The log of total digestive tract length was positively correlated with log body mass, GEI and DEI at day 28 (body mass:  $r^2=0.267$ ,  $P<0.05$ , Figure 6; GEI:  $r^2=0.237$ ,  $P<0.05$ , Figure 7; DEI:  $r^2=0.399$ ,  $P<0.01$ , Figure 8).

There was no significant difference in gizzard mass between the 10 °C and 30 °C birds at the end of the experiment (ANCOVA, wet mass with content,  $F_{1,13}=0.706$ ,  $P>0.05$ ; wet mass without contents,  $F_{1,13}=0.396$ ,  $P>0.05$ , dry mass,  $F_{1,13}=0.434$ ,  $P>0.05$ , Figure 5).

However, the mass of the small intestine changed significantly (ANCOVA, wet mass with contents,  $F_{1,13}=7.158$ ,  $P<0.05$ ; wet mass without contents,  $F_{1,13}=13.491$ ,  $P<0.01$ ; dry mass,  $F_{1,13}=15.569$ ,  $P<0.01$ , Figure 5). Small intestine wet mass with contents, wet mass without contents and dry mass were 30.1%, 35.0% and 36.3%

lower, respectively, in the 30 °C group than in their 10 °C counterparts.

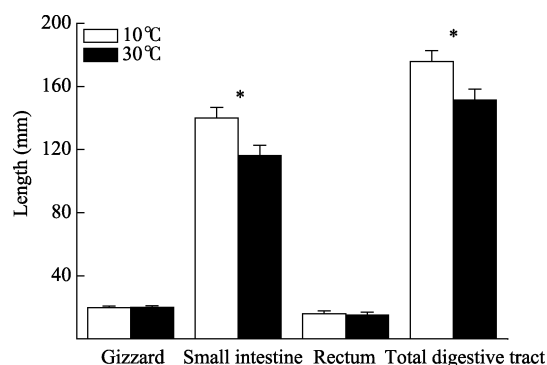


Figure 4 Effect of temperature acclimation on digestive tract length in the Chinese bulbul. Data are presented as mean $\pm$ SEM; \*:  $P<0.05$

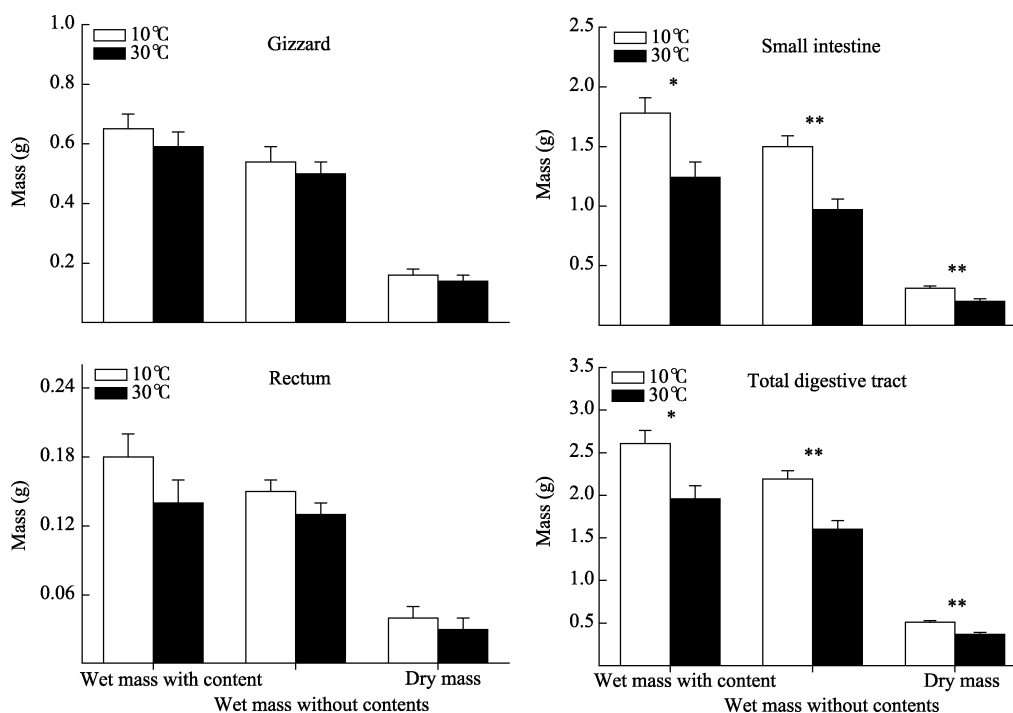


Figure 5 Effect of temperature acclimation on digestive tract mass in the Chinese bulbul

Data are presented as mean $\pm$ SEM. \*:  $P<0.05$ ; \*\*:  $P<0.01$ .

No significant difference in rectal mass between 10 °C and 30 °C birds was evident at day 28 (ANCOVA, wet mass with contents,  $F_{1,13}=1.832$ ,  $P>0.05$ ; wet mass without contents,  $F_{1,13}=1.311$ ,  $P>0.05$ , dry mass,  $F_{1,13}=0.484$ ,  $P>0.05$ , Figure 5).

However, bulbuls in the 30 °C group had a lighter digestive tract overall than 10 °C birds (ANCOVA, wet mass with contents,  $F_{1,13}=7.580$ ,  $P<0.05$ ; wet mass without contents,  $F_{1,13}=15.858$ ,  $P<0.01$ ; dry mass,  $F_{1,13}=16.275$ ,  $P<0.01$ , Figure 5). The log of wet and dry mass of the complete digestive tract was positively

correlated with log body mass, GEI and DEI at day 28 (body mass, wet mass:  $r^2=0.462$ ,  $P<0.01$ ; dry mass:  $r^2=0.626$ ,  $P<0.001$ , Figure 6; GEI, wet mass:  $r^2=0.635$ ,  $P<0.001$ ; dry mass:  $r^2=0.726$ ,  $P<0.001$ , Figure 7; DEI, wet mass:  $r^2=0.615$ ,  $P<0.001$ ; dry mass:  $r^2=0.712$ ,  $P<0.001$ , Figure 8).

## DISCUSSION

Our results suggest that ambient temperature had significant effects on the body mass, GEI, FE, and DEI of

Chinese bulbuls, all of which decreased significantly in birds acclimatized to 30 °C. These birds also underwent a significant decrease in the length and mass of the digestive tract.

Ambient temperature is known to have significant effects on many morphological physiological and biochemical parameters of birds, including body mass (Tieleman et al, 2003; Cooper, 2007; McKechnie et al, 2007; Zheng et al, 2008a). Our body mass results were consistent with those of previous reports on the response of Chinese bulbuls to seasonal changes (Zhang et al, 2008; Zheng et al, 2008b, 2010). We found that bulbuls acclimatized to 10 °C maintained a relatively constant body mass but that those acclimatized to 30 °C decreased in body mass over the four week experimental period, and by the end of the experiment the body mass of these birds was 7.5% lower than that of those kept at 10 °C (Figure 1A). The decrease in the body mass of bulbuls acclimatized to 30 °C reflected an imbalance between energy intake and expenditure, a hypothesis supported by changes in other parameters, such as GEI, DEI, and the length and mass of the digestive tract.

Environmental temperature can alter the energy intake of birds. Cold winter temperatures will increase energy consumption because of the increased need for energy to maintain body temperature. Warmer temperatures on the other hand decrease energy requirements because less heat needs to be produced to maintain body temperature (Cain, 1973; Syafwan et al,

2012). There is marked variation across avian species in the reported energy budgets associated with acclimatization or acclimation to changing ambient temperatures (Salvante et al, 2010; Syafwan et al, 2012). For example, the GEI of warm (38 °C) acclimated Muscovy ducks *Cairina moschata* decreased by 49% relative to that of control (20 °C) birds, and that of cold (−10 °C) acclimated ducks increased by 62% relative to that of control birds in an 11 hour photoperiod (Cain, 1973). Similarly, the GEI of summer acclimatized Elliot's pheasants *Syrnaticus ellioti* was 29% and 34% lower than that of autumn and winter acclimatized birds, respectively (Lou et al, 2003). This suggests that changes in GEI and DEI are a common response to variation in ambient temperature for small birds and mammals in both warm and temperate habitats (Stokkan et al, 1986). We found that Chinese bulbuls acclimatized to 30 °C underwent a 47% decrease in GEI, and a 45% decrease in DEI, relative to those kept at 10 °C. These changes in GEI and DEI were positively correlated with body mass (Figure 3). These results suggest that bulbuls acclimatized to 30 °C experienced an obvious decrease in energy requirements. One could predict that, if different physiological systems have to compete for energy, the combination of these energetically challenging processes would affect heat production, as we found. Chinese bulbuls acclimatized to 30 °C for four weeks decreased their basal metabolic rate (BMR), liver, kidney and small intestine mass, and mitochondrial state-4

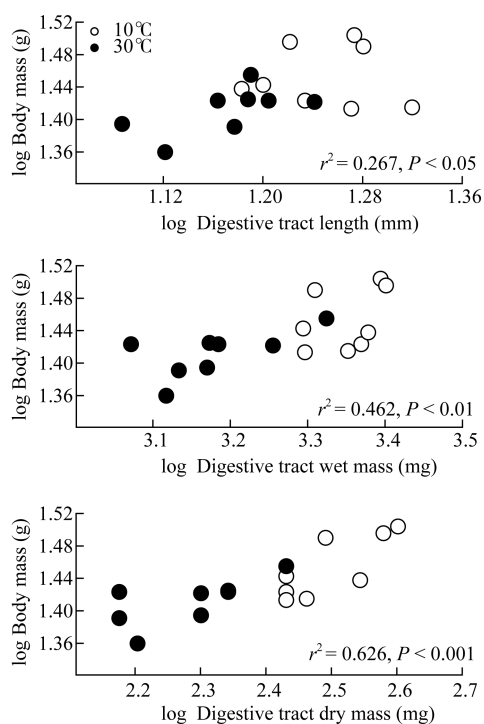


Figure 6 Least square regression of length and wet and dry mass of the digestive tract as dependent variables of body mass in Chinese bulbuls acclimated to 30 °C or 10 °C

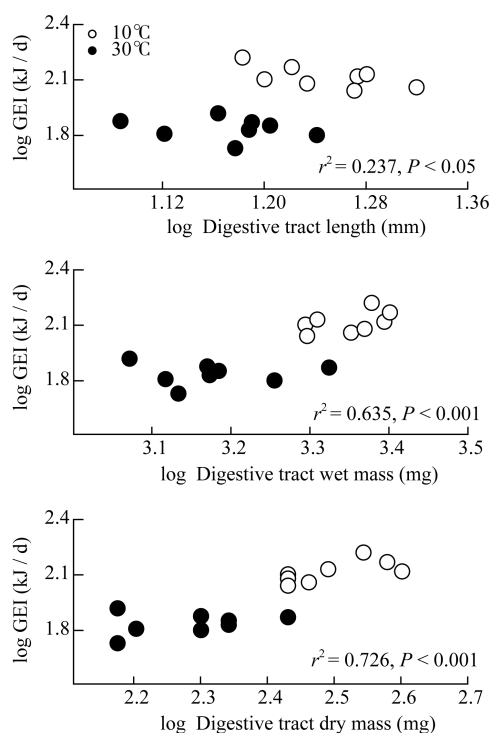


Figure 7 Least square regression of length and wet and dry mass of the digestive tract as dependent variables of GEI in Chinese bulbuls acclimated to 30 °C or 10 °C

respiration and cytochrome c oxidase (COX) activity in liver and muscle, compared to those kept at 10 °C (Zheng et al., 2013). In view of the mass-specific energy metabolism of these organs and/or tissues, the observed decreases in body mass, GEI and DEI were not surprising.

Environmental temperature also influences morphological and physiological functions of the digestive tract in birds (Williams & Tieleman, 2000; Starck & Rahmaan, 2003; Karasov, 2011; Karasov et al., 2011). During cold conditions, when energy demands increase, small birds may increase their energy intake, which simultaneously compromises their digestive-efficiency unless they also undergo associated changes in gut size, enzyme activity, nutrient uptake and/or food transit time (McKinney & McWilliams, 2005; Karasov et al., 2011). For example, warm (35 °C) acclimated woodlarks, skylarks, spike-heeled larks *Chersomanes albobasata* and Dunn's lark underwent a respective 21%, 22%, 9%, and 24% decrease in stomach mass, and a 21%, 23%, 23%, and 21% decrease in intestinal mass, relative to that of cold (15 °C) acclimated conspecifics (Tieleman et al., 2003). Similarly, summer acclimatized spruce grouse *Canachites canadensis* had a 44% lower ventricular mass and a 21% shorter small intestine than winter acclimatized birds (Pendergast & Boag, 1973). According to Sibly's (1981) model of optimal digestion, one of the advantages of increasing digestive tract size is that it allows an increase in the mean retention time of digesta, thereby increasing digestibility if the ingestion rate is constant. Alternately, it allows a constant mean retention time of digesta, which maintains digestive efficiency if the ingestion rate increases. Our results showed that bulbuls acclimatized to 30 °C decreased the size of their gastrointestinal tracts relative to those bulbuls kept at 10 °C. The significant, positive relationship between log body mass, GEI and DEI (Figure 6, Figure 7 and Figure 8), suggests that this was an adaptive response to decreased body mass and energy intake.

In conclusion, Chinese bulbuls in the 30 °C group had lower body mass, GEI and DEI relative to those in

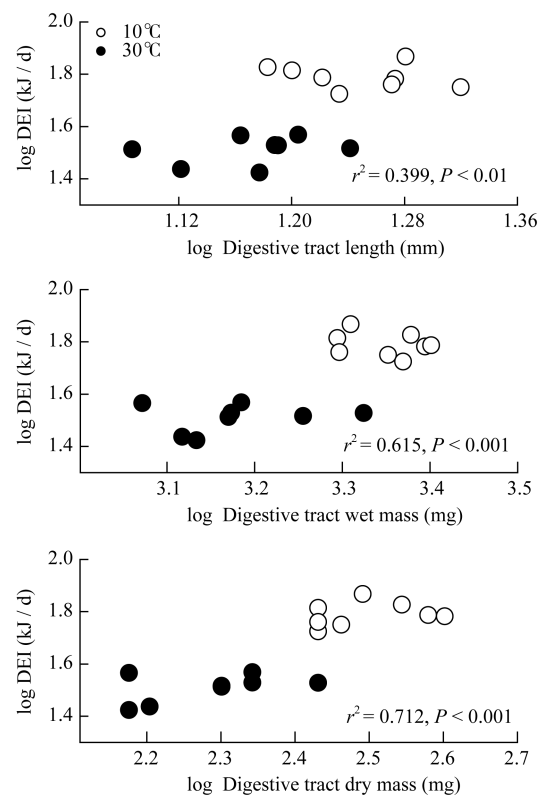


Figure 8 Least square regression of length wet and dry mass of the digestive tract as dependent variables of DEI in Chinese bulbuls acclimated to 30 °C or 10 °C

the 10 °C group. The length and wet and dry mass of the digestive tract of bulbuls were also lower in the 30 °C group and had significant positive relationships with body masses, GEI and DEI. Chinese bulbuls reduced their energy demands at high temperatures by decreasing their GEI and DEI, and digestive tract size.

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# Acoustic characteristics of eight common Chinese anurans during the breeding season

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**Abstract:** Anurans often have species-specific vocalizations. To quantify and compare the characteristics of anuran calls in Gutianshan National Nature Reserve, Zhejiang Province, we recorded the advertisement calls of eight species belonging to four families (Ranidae, Microhylidae, Megophryidae and Bufonidae) from June to September 2012 using Sony ICD-FX8 IC recorders. All recordings were analyzed using the “Praat” software. Five acoustics parameters were measured, including temporal traits (call duration, number of notes or pulse number/call) and spectral traits (fundamental frequency, the first three formants and dominant frequency). The characteristic parameters of *Microhyla ornate* and *Fejervarya limnocharis* calls were different as were the calls of some populations of the same species recorded in different regions. The advertisement calls of the eight species were specific. Our study has provided a useful reference for identifying the calls of some common Chinese anurans.

**Keywords:** Anurans; Advertisement calls; Acoustic characteristics

Sound communication is one of the most important means of animal communication. Research on sound communications in insects, birds and mammals indicate that there are intimate connections between sound, social activities and behaviors (Li et al, 1993; Shen et al, 2007; Zhou et al, 2004). Anurans are the largest amphibian taxon and have a mate recognition system based on advertisement call (Marshall, 2005; Stuart et al, 2006). Vocalizations are therefore a useful diagnostic feature for identifying anuran species.

There is a large body of research on anuran vocalizations, including many studies of advertisement calls (McLeod et al, 2001). In some species, the quality and structure of male calls influence the outcomes of male-male contests and female choice (Bee, 1999; Pröhl, 2003; Yu & Zheng, 2009). Female always prefer male with calls of lower fundamental frequency, more variable calls or that can sustain calling longer than their competitors (Howard & Young, 1998; Morris & Yoon, 1989; Pröhl, 2003; Ryan & Drewes, 1990). Call surveys are a widely used and accepted monitoring technique for predicting anuran calling activity (Kirlin et al, 2006; Steelman & Dorcas, 2010).

A three-parameter model for classifying anurans on the basis of their advertisement calls correctly identified

species with an accuracy of >70% (Gingras & Fitch, 2013). Because of their highly species-specific character, some studies have used calls to evaluate relationships between different species and to identify cryptic species (Abrunhosa et al, 2005; Heyer & Barrio-Amorós, 2009; Nunes et al, 2007; Roy & Elepfandt, 1993; Wychwerley et al, 2002; Yu & Zheng, 2009).

To date, research on anuran vocalizations in China has been relatively limited. So far, aspects of the calls of more than 30 species have been analyzed, including pulse rate, dominant frequency, fundamental frequency and formant frequency (Chen et al, 2011; Cui et al, 2011; 2012; Huang et al, 1982; Jiang et al, 1995; Jiang et al, 2002; Matsui & Wu, 1994; Shen et al, 2008; Xu et al, 2005; Yu & Zheng, 2009; Wang et al, 2012; Wei et al, 2011; 2012; 2013). In recent years, research has focused more on the calls of single

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species in one location, rather than addressing differences between the calls of different species, or regional differences in the calls of the same species (Chen et al, 2012; Cui et al, 2011, 2012; Wei et al, 2011). Although the sound analysis software adopted in different studies was various, the results were still relatively consistent. However, the way to define parameters, such as pulse, note, etc., would make it difficult to compare the results from individual research groups.

We here present the results of an analysis of the vocalizations of the advertisement calls of eight common Chinese anurans based on the standard set of parameters used by Cui et al (2012). The species of anurans are extremely rich in China, but the basic datum of the vocalizations of the anurans advertisement calls are limited. This study aims to expand the datum of the vocalizations of anurans communication calls, and to provide a useful reference for identifying the calls of some common Chinese anurans.

## MATERIALS AND METHODS

### Research objectives

Our primary objective was to record, characterize and compare the advertisement calls of eight anuran species, *Bufo melanostictus* (Bufonidae), *Xenophrys boettgeri* (Megophryidae), *Microhyla ornate* (Microhylidae), *Odorrana schmackeri*, *Limnonectes fujianensis*, *Hylarana adenopleura*, *Fejervarya limnocharis* and *Hylarana guentheri* (Ranidae). *X. boettgeri*, *H. adenopleura*, *L. fujianensis* and *O. schmackeri* were recorded in the Gutianshan National Nature Reserve (N29.14°, E118.05°), Quzhou City, Zhejiang Province. *H. guentheri* and *B. melanostictus* were mainly recorded at Daluo Mountain (N27.93°, E120.71°), Chashan Town, and Wenzhou City. *F. limnocharis* and *M. ornata* were recorded in both locations.

### Recording methods

Anuran advertisement calls were recorded between 19:00 and 05:00 from June to September 2012, which was the most active period of the day and part of the year in which the species under investigation. Calls were recorded for 5–30 min by Sony IC recorders (ICD-FX8) without external directional microphone which were usually held 15–20 cm away from the animal being recorded. After the calls of an individual animal were recorded, the subject was photographed to aid subsequent identification (Cui et al, 2012).

### Analysis of call Parameters

*Cooledit Pro 2.0* was used to reduce background noise at a sampling rate of 44.1 kHz and 16 bit resolution after which *Praat 5.3.32* was used to analyze call traits

(Boersma & Wecnick, 2006). Each individual sound file for each species contained at least 10 calls. The acoustic parameters of calls measured including temporal properties, such as call duration, number of notes and note duration, as well as spectral properties, including dominant frequency (DF), fundamental frequency (F0) and harmonics (F1–F3). The Fast Fourier Transformation (FFT) procedure which the frame size is 512 in Matlab software (R2010b) was used to obtain the main peak, or dominant frequency, of each call. We didn't record the acoustic pressure which is affected by the distance of recording. Because of the large variation in number of notes and call duration between species, we analyzed and compared either the first or last note of the call of different species. Data were analyzed with independent-samples *t*-test (*t*-test). Statistics are presented as means  $\pm$  *SD*, the threshold of statistical significance was set at  $\alpha=0.05$ . Statistical analyses were performed by SPSS 13.0.

## RESULTS

Parameters of the calls of the eight species are in Table 1.

### Call Trait Analysis

#### Bufonidae

##### *Bufo melanostictus* (Figure 1a)

Males of this species have an internal vocal sac and call continually at night. Advertisement calls are composed of single notes which increase in amplitude during the call sequence. Variation in call duration was high (mean=47.22, *SD*=49.73, Table 1), which is probably a consequence of the small sample size (*n*=3).

#### Megophryidae

##### *Xenophrys boettgeri* (Figure 1b)

Based on analysis of 140 calls from six individuals, notes are generally frequent but short and the call duration is always long. The fundamental frequency (F0) was the highest among the eight species recorded (Table 2) and higher than the first (F1) frequency.

#### Microhylidae

##### *Microhyla ornate* (Figure 1c)

Males of this species have an internal vocal sac under a single pharyngeal sac. One hundred and twenty recordings of seven individuals from Gutianshan indicate that each call has a relatively long duration of up to 12 notes (mean=12.34, *SD*=2.20). However, 172 recordings of 11 individuals of the same species at Wenzhou (Table 2) indicate a pulse number up to 15 (*SD*=3.87). In addition, the calls of different individuals were found to have either one of two different dominant frequencies (DF) (Table 1).

Table 1 Descriptions of acoustic characteristics of eight anuran species (All data were not adjusted by temperature)

Species	Call duration/s	Note numbers/call	Note duration/s	Dominant frequency (DF, Hz)	Fundamental frequency (F0, Hz)	F1 (Hz)	F2 (Hz)	F3 (Hz)
<i>Bufo melanostictus</i> * (n=3)	6.14±4.86	47.22±49.73	0.14 ± 0.06	1758.50±72.24	874.08±45.28	1545.70±83.66	1806.14±68.21	2661.98±282.54
<i>Xenophrys boettgeri</i> (n=6)	6.26±1.97	25.99±8.64	0.08 ± 0.01	3468.94±129.54	1727.96±128.40	1443.28±176.37	2718.41±297.20	3381.73±188.51
<i>Microhyla ornate</i> (n=7)	0.26±0.06	12.34±2.20**		577.44±55.67 2221.84±235.35	1276.46±262.79	1425.32±69.17	2162.59±230.44	2795.22±228.84
<i>Hylarana adenopleura</i> (n=14)	0.64±0.21	2.15±0.79	0.18 ± 0.02	577.44±55.67 2221.84±235.3	309.85±22.34	813.66±64.38	1882.94±132.10	2361.65±141.08
<i>Limnonectes fujianensis</i> (n=3)	0.30±0.04	6.15±0.76**		531.09±50.76	512.59±15.04	667.29±25.12	1318.65±106.72	2186.64±235.71
<i>Odorrana schinackeri</i> (n=10)	0.04±0.02	1.64±0.52	0.03 ± 0.01	3479.14±406.70	908.45±76.18	1623.77±370.67	2457.01±464.83	3185.98±388.66
<i>Fejervarya limn-ocharis</i> (n=6)	0.16±0.08	1.33±0.49	0.07 ± 0.01	1456.28±83.55 2893.48±115.92	1458.94±65.72	1374.81±87.23	2108.96±422.67	2794.47±241.51
<i>Hylarana guentheri</i> * (n=3)	0.15±0.01	1.00±0.00	0.15 ± 0.01	354.34±6.87	324.38±35.49	618.56±120.47	1189.61±147.98	1679.43±191.43

\*: Calls recorded at Wenzhou, the remaining six species were recorded at Gutianshan; \*\*: Pulse number/call; The first note of *Xenophrys boettgeri* and *Hylarana adenopleura* were used to analyze the last five parameters excluding call duration, note number and note duration; The corresponding parameters of *Bufo melanostictus* were determined by the analysis of the last note; Data are presented as mean±SD; Given that in some species there are two apparent dominant frequencies, for the parameters of dominant frequency, two values were present.

## Ranidae

### *Hylarana adenopleura* (Figure 1d)

Males of this species have paired internal pharyngeal vocal sacs and calls that are relatively easy to identify. One hundred and twenty-one calls from 14 individuals indicate that different individuals have either one of two dominant frequencies (DF), one at 577 Hz and the other at about 2 221 Hz. The fundamental frequency (F0) was the lowest among the eight species recorded (Table 1).

### *Limnonectes fujianensis* (Figure 1e)

Males of this species don't have vocal sacs and occasionally call during the day. The call analyzed was chosen from 33 recordings of 3 individuals. Each note lasted 317~351 ms and the dominant frequency (DF), fundamental frequency (F0) and first frequency were close (Table 1). However, it was very difficult to

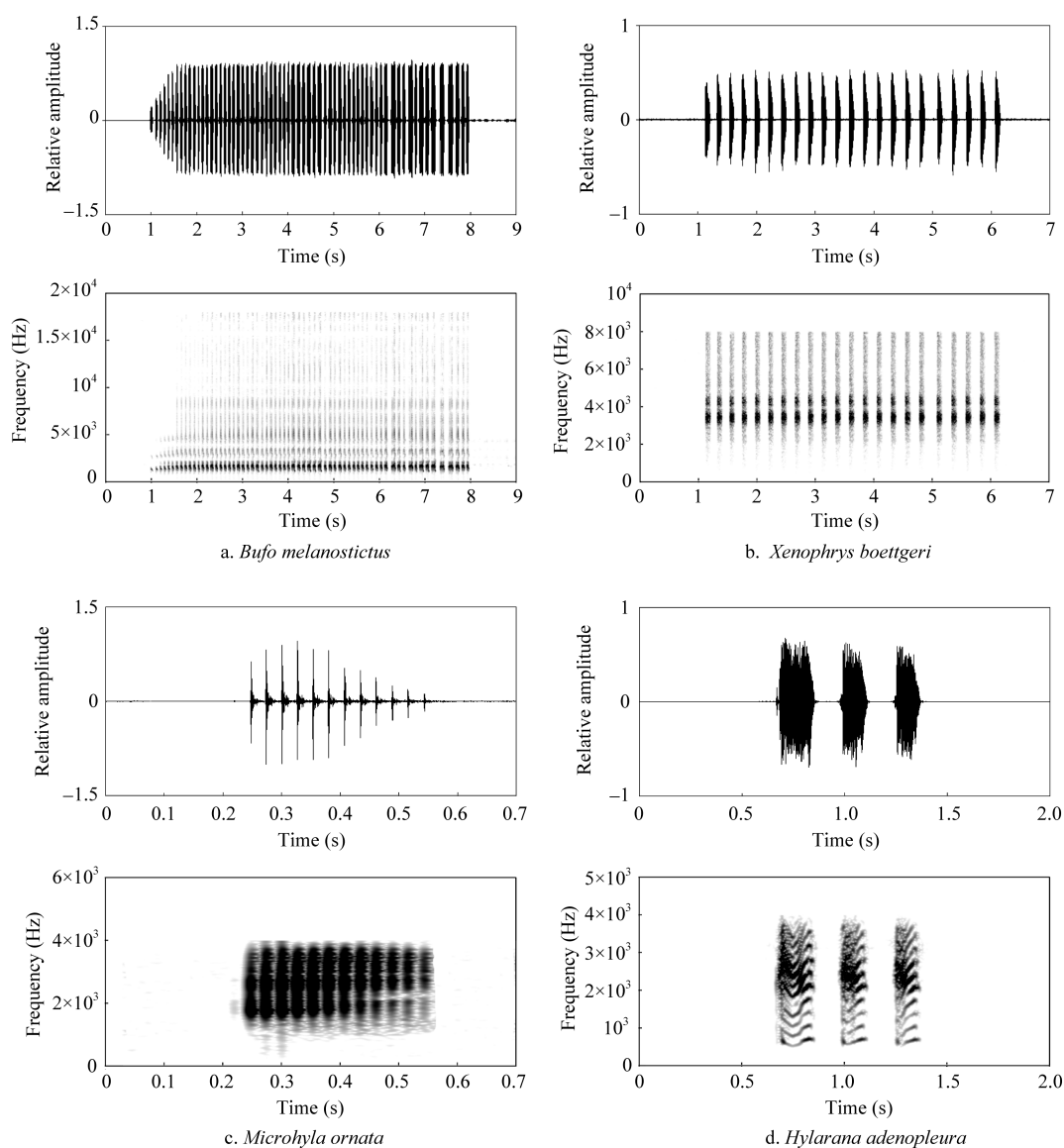
measure the pulse number because the notes were weak.

### *Odorrana schmackeri* (Figure 1f)

During the peak of the breeding season males of this species call *en mass* beginning after nightfall. Based on analysis of 98 recordings from 10 individuals, most male calls had a prelude which was relatively easy to record. This species had the highest dominant frequency (DF) among those recorded (Table 1).

### *Fejervarya limnocharis* (Figure 1g)

The call of this species had fewer notes than the other species and was relatively short. Males and females of this species congregate near water and call day and night during the breeding season. As in *H. adenopleura* and *M. Ornate*, two distinct dominant frequencies (DF) were detected in different individuals (DF), one of which was close to F0 (Table 1).



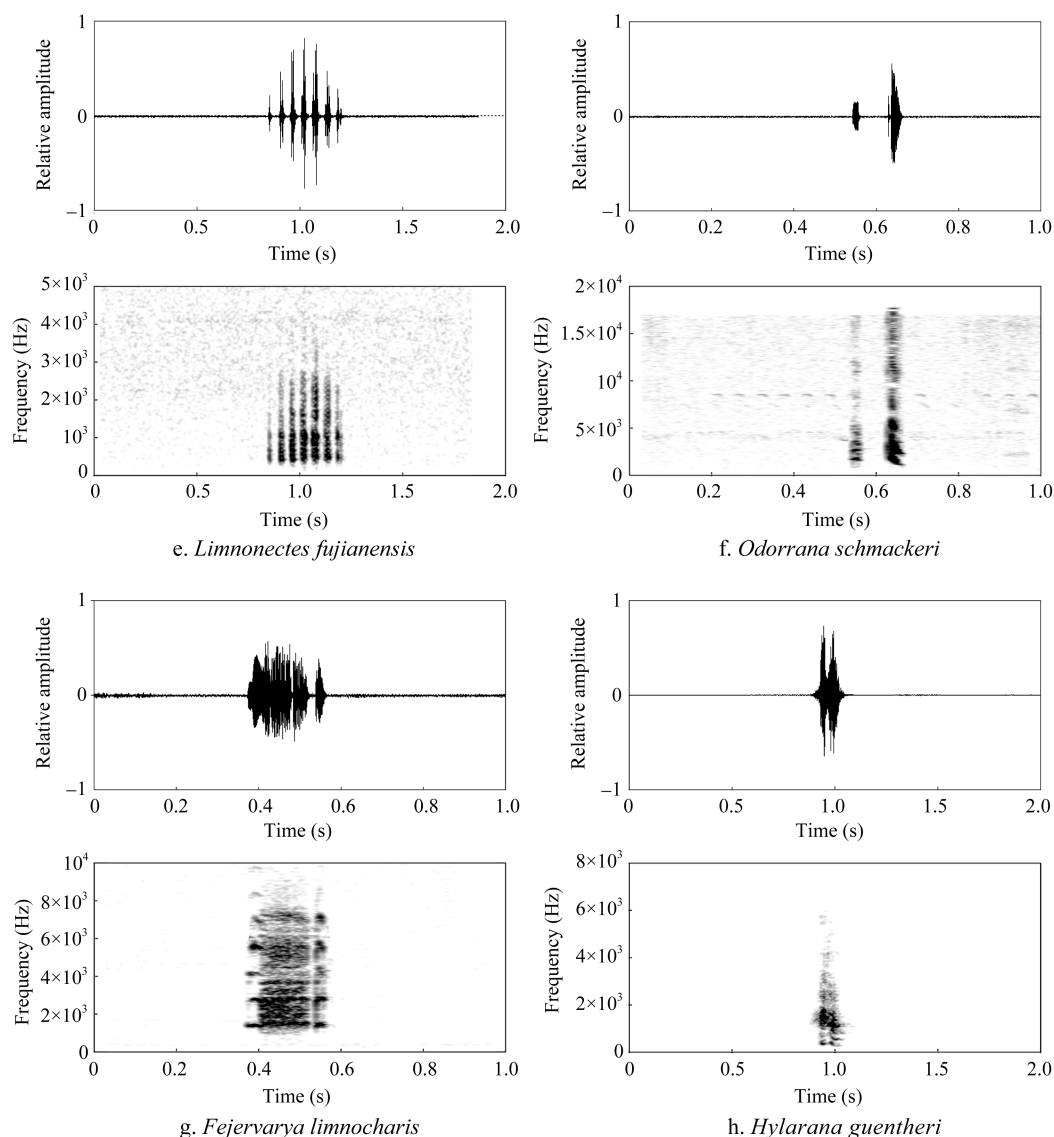


Figure 1 Oscillograms and spectrograms of mating calls of eight anuran species recorded during breeding season

a: *Bufo melanostictus*; b: *Xenophrys boettgeri*; c: *Microhyla ornata*; d: *Hylarana adenopleura*; e: *Limnonectes fujianensis*; f: *Odorrana schmackeri*; g: *Fejervarya limnocharis*; h: *Hylarana guentheri*; Software was Praatt 5.3.32 and frequency range is 0~5 500 Hz

### *Hylarana guentheri* (Figure 1h)

Males of this species have a pair of internal vocal sacs and call day and night during the breeding season. Its single-note, bark-like call was easy to identify and had the lowest first frequency (F1) among the species recorded (Table 1).

## DISCUSSION

### Species Identification

Many Chinese anuran species are sympatric and have therefore evolved species-specific vocalizations to attract conspecific females. The species-specific nature of anuran advertisement calls means that these calls can provide a useful means of identifying different species.

We found large differences in the calls of different species in features such as call duration, note number, fundamental frequency, dominant frequency and the first three formants. Some of these differences can be attributed to species-specific differences in the morphology and structure of the vocal apparatus (Xiong et al, 2010). For example, *F. limnocharis*, *X. boettgeri* and *M. ornata* all have a single external vocal sac, but differ in call duration and number of notes. As in *X. boettgeri*, the calls of different individuals of these two species appear to have two different dominant frequencies, one of which is close to the fundamental frequency and the other double the fundamental frequency.

The call durations of *H. adenopleura*, *H. guentheri*

and *O. schmackeri* were shorter than those of the other species and had relatively fewer and more distinct notes. *Bufo melanostictus* has a long call duration with many notes, a low fundamental frequency and a high dominant frequency.

Due to the lack of a vocal sac, advertisement calls of male *L. fujianensis* were relatively weak, and the dominant and fundamental frequency of this species were lower than in the other remaining species.

There are obvious differences in the morphology and structure of vocal apparatus between frogs and toads. For example, in the Chinese forest frog *Rana chensinensis* the structure of the *pars lateralis* and middle of the vocal cords resembles the letter “T”, whereas in the toad *Bufo raddei* it looks more like the letter “V” (Zhang et al, 2012). Another influence on the sounds produced by different species is the number of throat muscles (Zhang, 1988). There are four types of throat muscle arrangements in Chinese anurans; two pairs, three pairs, four pairs and five pairs (Zhang, 1988). The *Ranidae* have four pairs of muscles whereas the *Bufonidae* have only three pairs. The number of throat muscles possessed by the *Megophryidae* remains unknown (Zhang, 1988).

It is likely that species-specific differences in call frequency and intensity often reflect such differences in the vocal apparatus between different species. Determining the reasons for these differences will require anatomical investigation of the male vocal apparatus.

### Geographic Variation in Calls within the same Species

We also found evidence of geographic variation in calls of the same species. Xu (2005) found evidence of geographic variation in the calls of *Rhacophorus dugrititei* and *Rhacophorus chenfui*. Wei (2012) found differences in the calls of Chinese and overseas populations of *B. melanostictus*.

Comparing calls of *M. ornata* recorded in Gutianshan

and Wenzhou with those of the same species recorded by Jiang (1995) in Hangzhou and Yishan, and by Wei (2013) in Lishui (Table 2), we found that, although the amplitude and duration of the syllable were similar between locations, the call duration was higher at Yishan (2.4 s, Table 2). Furthermore, call duration and peak frequency differed between locations.

The calls of *M. ornata* recorded in Wenzhou, Gutianshan and Yicheng (Anhui) had two high power peaks, whereas those recorded in Hangzhou and Lishui had only one. There were also significant differences in call duration, note number, fundamental frequency, dominant frequency and first formant between *M. ornata* calls recorded at Gutianshan and Wenzhou (*t*-test,  $P<0.05$ , Table 2, Figure 2). There was, however, no significant difference in the second and the harmonic (*t*-test,  $P>0.05$ ). As the same results were reported by Wei et al. (2013), we think it is likely that there is geographic variation in the calls of *M. ornata*. However, the technical differences in sound recording and analyzing may have caused the variations of acoustic characteristic parameters.

We also compared the calls of *F. limnocharis* we recorded at Gutianshan and Wenzhou with those recorded by Jiang et al (1995) in Hangzhou and Shengsi (Figure 3). Although most frequency character of the calls from these four populations were similar, there were still some discrepancies in some call frequency. For example, the dominant frequency (DF) in the Hangzhou population was much lower than that in the other populations. Moreover, significant geographical variation was apparent in call duration, note number, fundamental frequency and the first formant (*t*-test,  $P<0.05$ ).

Anurans emit a variety of sounds during the breeding season. In most anuran species, only males call, only in a few species the females also vocalize. For example, female Emei music frogs, *Babina daunchina*, call to stimulate inter-male competition (Cui et al, 2010).

**Table 2** Acoustic features of *Microhyla ornata* recorded at different sites

	Site	Pulse number/call	Call duration (s)	Fundamental frequency (F0, Hz)	DF (kHz)	F1 (Hz)
<i>Microhyla ornata</i>	Hangzhou (Jiang et al, 1995)	NA	0.31±0.02	NA	1.36±0.02	NA
	Yicheng (huang et al, 1982)	NA	2.40	NA	2.80-2.90	NA
	Lishui (Wei et al, 2013)	NA	0.30±0.03	NA	2.50±0.50	NA
	Gutianshan (this work)	12.34±2.19	0.26±0.05	1276.46±262.79	1.73±5.74 2.80±0.22	1425.32±69.17
	Wenzhou (this work)	15.66±3.87	0.27±0.03	1359.71±12.95	1.31±0.05 2.72±0.12	1369.25±40.35

Data are presented as mean±SD; The different methods of sound recording and analyzing would cause the variations of acoustic characteristic parameters.

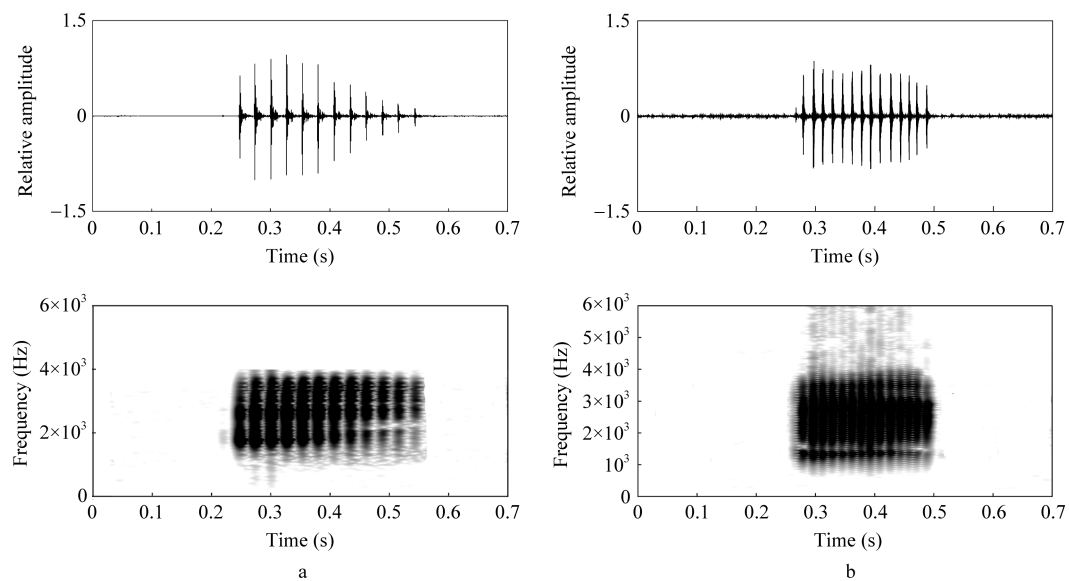


Figure 2 Differences in calls of *Microhyla ornata* recorded at (a) Gutianshan and (b) Wenzhou

a: Recorded at Gutianshan (the inter-note intervals are average and the envelope reduces gradually); b: Recorded at Wenzhou (the inter-note intervals are smaller and the envelope is linear)

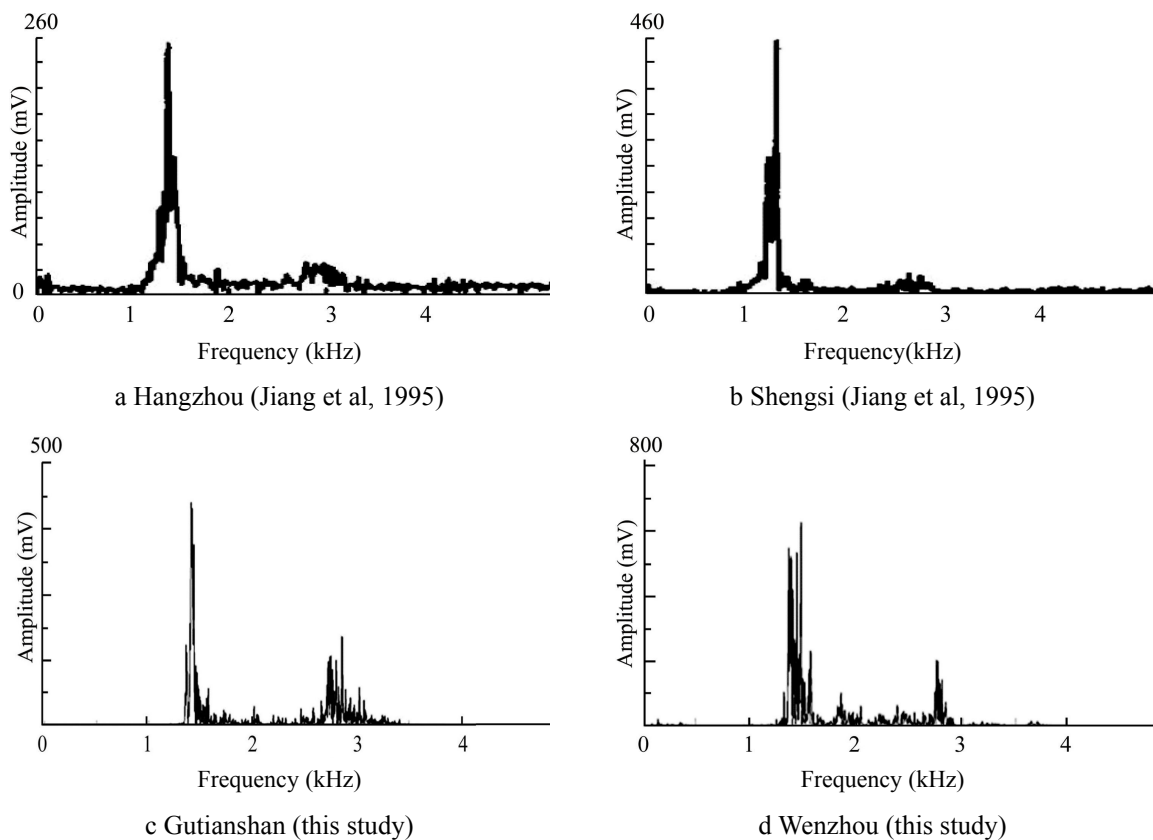


Figure 3 Frequency spectrum analysis of calls of *Fejervarya limnocharis* recorded at four different locations

Anurans' calls can convey quite complex information. For example, male *B. daunchina* can convey not only the possession of, but the quality of a

cave territory to females which preferentially mate with males holding higher quality territories (Cui et al, 2012). On the other hand, environmental conditions such as

temperature can also cause individual variation in vocalization within a species or population. For example, Chen et al (2012) found that call duration and syllable length were negatively correlated with environmental temperature. In addition, individual variation in body mass and body size can also affect call traits (Tárano, 2001; Wang et al, 2012).

Further research is required to clarify the relationship between calls and behaviors. For example, we found that male *L. fujianensis* protected their partner's eggs during the breeding season. Therefore,

the possibility that male's may vocalize differently when they are protecting eggs as opposed to attract mates requires further investigation. The significance of the two types of dominant frequencies in different individual *X. boettgeri*, *M. ornate* and *H. adenopleura* also requires further investigation.

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## Four new records of fish species (Cypriniformes: Nemacheilidae, Balitoridae; Characiformes: Prochilodontidae) and corrections of two misidentified fish species (Tetraodontiformes: Tetraodontidae; Beloniformes: Belonidae) in Yunnan, China

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**Abstract:** In this study, six fish species of five families are reported for the first time from Yunnan Province, China. The nemacheilid *Schistura amplizona* Kottelat, 2000 is reported from the Luosuojiang River and Nanlahe River subbasins, Mekong basin; the prochilodontid *Prochilodus lineatus* (Valenciennes, 1837), the balitorid *Vanmanenia serrilineata* Kottelat, 2000, and the tetraodontid *Monotretus turgidus* Kottelat, 2000, from Nanlahe River subbasin, Mekong basin; the balitorid *Beaufortia daon* (Mai, 1978), and the belonid *Xenentodon canciloides* (Bleeker, 1854), both, from Black River subbasin, Red River basin. The freshwater puffer *M. turgidus* and the needlefish *X. canciloides* have been previously misidentified as *Tetraodon leiurus* (Bleeker, 1950) and *Tylosurus strongylurus* (van Hasselt, 1823), respectively.

**Keywords:** New record; Misidentification; Mekong basin; Red River; Yunnan

Yunnan Province is located in the Southwest within the People's Republic of China. Its name refers to its location south of the Yunling Mountain range. It shares international border with Myanmar in the West and Southwest, with Laos and Vietnam in the South; national borders with Xizang Autonomous Region to the Northwest, with Sichuan Province to the North, Guizhou Province to the East, and with Guangxi Province to the Southeast. Yunnan can be roughly divided into two different geological formations; the Yunnan-Guizhou plateau, a limestone plateau with karstic landscapes in the East, and deep mountainous gorges with basically north to south running major rivers in the West such as the Salween and Mekong. Due to its geological peculiarities the respective climate is highly variable ranging from alpine in more than 5000 m high northern plateaus to subtropical in lowland Xishuangbanna close to the Laotian border (Wikipedia contr., 2013).

The high diversity in terms of geology, topography and climate boost biodiversity as well as the diversity of fishes populating Yunnanese water bodies. The seminal monograph about the Yunnanese ichthyofauna, *The Fishes of Yunnan, China*, has been compiled by Chu &

Chen et al in 1989, respectively 1990 for the second volume, giving 226 species and subspecies accounts in the first volume plus an additional 173 in the second. Through extensive fieldwork and re-evaluation of institutionally stored lots the number of Yunnanese fish species is growing (for e.g. Endruweit, 2011; Zheng et al, 2012). Recent fieldwork has unveiled six fish species of five families not yet known to occur in Yunnan; two of those have been previously misidentified. Species are herein diagnosed and discussed.

## MATERIALS AND METHODS

Counts, measurements, and terminology of morphological features follow Kottelat & Freyhof (2007: 15-21). One exception is that lateral line scales are counted in total including those pored located on the caudal fin. Measurements were taken with a dial caliper to the nearest 0.1 mm; counts were taken from the left

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side of a specimen wherever possible, in some cases with the help of a magnifying glass and a monocular microscope XSP-06. Examined specimens had been placed in the repository collection of the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences, and in the collection of the author (EPC).

## RESULTS

### *Schistura amplizona* Kottelat, 2000



Figure 1 *Schistura amplizona*, KIZ2012004193, 68.4 mm SL, lateral view, reversed, scale bar=5 mm

### Examined material

*Schistura amplizona* Kottelat, 2000, KIZ 2010003103-9, EPC 0991-3, 10 ex., 50.1–73.8 mm SL, Menglun market, Luosuojiang River subbasin, Mekong basin, Xishuangbanna, Yunnan, China, collected by M. Endruweit, 2010-12-30 (considered for meristics and morphometrics); KIZ 2012004198-9, 2 ex., 63–71 mm SL, Luosuojiang River subbasin, Mekong basin, Xishuangbanna, Yunnan, China, collected by M. Endruweit & Wang Jing, 2010-6-3; KIZ 2012004193-7, 5 ex., 48.6–68.4 mm SL, Mengla market, Nanlahe River subbasin, Mekong basin, Xishuangbanna, Yunnan, China, collected by M. Endruweit & Jing Wang, 2010-6-5.

### Diagnosis

*Schistura amplizona* is readily distinguished by its specific color pattern: Five to seven dark brown elongated blotches, much wider than interspaces and usually the widest at midline, superimposed by a broad, faint brown midlateral stripe on a yellowish body; blotches usually wider anteriorly than posteriorly; some blotches ovoid (autapomorphic characteristic); basal caudal bar prominent black, slightly oblique, restricted to the lower two-thirds; a black spot at the base of the upper principal caudal fin rays in some specimens; dorsal fin with a prominent dark brown horizontal blotch along the base; all fins with series of dark marks. Morphological characteristics are an elongate head and body; lower jaw without a notch; caudal-peduncle posteriorly with hard dorsal and ventral crests, 1.42–1.69 times longer than deep (Kottelat, 2000).

### Habitat and distribution

*Schistura amplizona* is herein reported from three locations; Menglun and Mengla markets and the

Nanxinghe River, Luosuojiang River subbasin, Mekong basin, around 3 km downstream of Mengxing village, Xishuangbanna, Yunnan. For detailed information about this biotope see Endruweit (2011). Kottelat (2001) lists the northern Laotian Nam Tha and Nam Youan rivers as the natural occurrence range. The Nam Youan River drains from Laotian Luang Namtha Province into Xishuangbanna and is then called Nanrunhe River. It is a left bank affluent to the Nanlahe River, in which watershed also Mengla is located and which is in turn a left bank tributary to the Mekong. The herein reported occurrence within the Luosuojiang River subbasin extends the natural occurrence range of *S. amplizona* towards North.

### Remarks

The examined species lots meet all characteristics required to identify *S. amplizona* despite a stouter caudal-peduncle of 1.25–1.41. In addition the basal caudal bar was not restricted to the lower two-thirds, but formed an almost complete bar, never reaching either dorsal or ventral extremities (e.g. KIZ 2012004193). The caudal bar proved to be resistant against fading in alcohol. Even when the dark brown blotches and all other color patterns started fading the basal caudal bar remained conspicuously intense black. The stouter caudal peduncle of 1.25–1.41 (vs. 1.42–1.69 in the original description) observed in the Luosuojiang River populations of *Schistura amplizona* may be seasonally and/or environmentally induced. It is regarded as an ecophenotypic variation.

### *Prochilodus lineatus* (Valenciennes, 1837)



Figure 2 *Prochilodus lineatus*, KIZ2012000191, 120.6 mm SL, lateral view, reversed, scale bar=10 mm

### Examined material

*Prochilodus lineatus* (Valenciennes, 1837), KIZ 2012000190-2, 3 ex., 120.6–141.7 mm SL, Mengla market, Nanlahe River subbasin, Mekong basin, Xishuangbanna, Yunnan, China, collected by M. Endruweit, 2011-1-2.

### Diagnosis

The three examined specimens match well the diagnosis and figure given in the revision of Castro &

Vari (2004): Caudal fin hyaline; lateral line scales 40–50; circumpeduncular scales 17–21; lower, posterior area of flanks with horizontal zigzag lines; transverse scales to dorsal origin 7–10; and predorsal scales 14–20.

Habitat and distribution.

Introduced species; Nanlahe River subbasin, Mekong basin, Xishuangbanna, Yunnan.

### Remarks

*Prochilodus lineatus* belongs to the order Characiformes, family Prochilodontidae. It is an alien species to China. It is originally described from the Rio de la Plata basin in Argentina. Its original range is limited to the Rio Paraná-Paraguay basin in Argentina, Brazil, Paraguay, and Uruguay (Castro & Vari, 2004). As a widespread species it possesses high ecophenotypic variation induced by abiotic and biotic environmental factors. Morphometric values may therefore differ tremendously between populations (Cabrera & Candia, 1964). The species attain a length up to 460 mm SL and is detritivorous. Chaloupkova et al (2010) reports *Prochilodus lineatus* in the Red River basin in Lang Son and Cao Bang Provinces, Vietnam, assumed to have escaped from aquaculture.

Three specimens (KIZ 2012000190–2) of *Prochilodus lineatus* were obtained off a large basket with a jumble of wild caught fishes. The seller, a local Dai women, confirmed that these specimens were cast netted within the Nanlahe River subbasin, a left bank tributary to the Mekong in Xishuangbanna. She also confirmed that this was not the first time to catch this species which entails that there is a self-sustaining population of *Prochilodus lineatus* in the Mekong basin in China.

### *Vanmanenia serrilineata* Kottelat, 2000



Figure 3 *Vanmanenia serrilineata*, KIZ2012004200, 64.9 mm SL, lateral view, scale bar=5 mm

### Examined material

*Vanmanenia serrilineata* Kottelat, 2000, KIZ 2012004200, 1 ex., 64.9 mm SL, Nanlahe River subbasin, Mekong basin, Xishuangbanna, Yunnan, China, collected by M. Endruweit & Jing Wang, 2010–6–4.

### Diagnosis

Kottelat (2001) gives two autapomorphic characters

for individuals larger than 25 mm SL of *Vanmanenia serrilineata*: An irregular, saw-toothed midlateral stripe; and 5 large saddles along back. The concerned specimen (KIZ 2012004200) meets both characters. In its original description a set of further synapomorphies is provided (Kottelat, 2000): Body deep (16.2%–20.3% SL); caudal-peduncle 1.2–1.4 times longer than deep, its depth 8.6%–9.7% SL; anus closer to tip of pelvic fin than to caudal fin origin; paired fins yellow with a series of dark marks in life; dorsal fin with 2–3 irregular rows of brown spots; branched pectoral fin rays 15–16; branched pelvic fin rays 8; lateral line pores 71–83. All these characters are present despite different number of branched pectoral and pelvic fin rays. The examined specimen has 14 branched rays in its left pectoral fin, while there are 15 branched rays in the right pectoral fin; branched pelvic rays were counted to 9.

### Habitat and distribution

The spot from which *Vanmanenia serrilineata* is reported is located around 40 km north of Mengla, Xishuangbanna, Yunnan; the stream is called Nanyanhe River and is a tributary of the Nanlahe River within the Mekong basin. The stream forms the border of a National Reserve with flocks of dense primary rainforest. During the dry season it was approximately 8 m wide and up to 1 m deep with the average being around 0.5 m deep. Clear water (16 °C; pH 8.1; 250 µs/cm; measured on 2011-1-1 at 11:00 am) ran over boulders and rocks with moderate to fast current. There were no vascular aquatic plants. The caught specimen represents the total yield across three samplings at this particular spot (January 2010, June 2010, January 2011). This is uncommon for *Vanmanenia* spp. They usually have large populations, sometimes being the predominant species in suitable habitats. There was no other balitorid species present. Syntopic species included the nemacheilids *Schistura macrocephalus* Kottelat, 2000 and *Pteronemacheilus meridionalis* (Zhu, 1982), the snakehead *Channa gachua* (Hamilton, 1822), and cyprinids such as *Barilius pulchellus* Smith, 1931, *Devario chrysotaeniatus* (Chu, 1981), *Poropuntius huangchuchieni* (Tchang, 1962), and *Scaphiodonichthys acanthopterus* (Fowler, 1934).

Kottelat (2000, 2001, 2009) reports *Vanmanenia serrilineata* from the Nam Ou and Nam Tha, subbasins to the Mekong in Phongsali and Luang Namtha provinces, northern Laos.

### Remarks

Geographically, Laotian Phongsali Province borders Xishuangbanna to the East and Luang Namtha Province to the Southwest. Hence, the confirmed occurrence of *V. serrilineata* in Xishuangbanna fills a zoogeographical gap.

The number of branched rays in pectoral and pelvic fin differ slightly from the original description. Unequal numbers in one specimen are rather common for balitorid loaches. With only 14 branched rays for the left pectoral fin the range given in the original description is not reached. Branched pelvic fin rays were counted to 9, equally in each fin. These slight deviations are based on just one specimen and must not be over-evaluated. Kottelat (2009: 17) erroneously listed this species as nemacheilid; *Vanmanenia* Hora, 1932 is a balitorid genus.

Zhou et al (2010) reported a *Vanmanenia* sp. from the upper Mekong in Yunnan based on 31 specimens collected from Laiyanghe River, Puer (5 ex.), Yangbiji River, Dali (20 ex.), Bijiang, Dali (2 ex.), and Youdianhe River, Changning (4 ex.). Kottelat (2000) gives body depth as 16.2%–20.3% of SL for *V. serrilineata* while Zhou et al give it to 17%–18.9% in *V. sp.* and 17.1%–18.9% in *V. striata*. This character was measured to 20%–22.3% in *V. tetraloba*. I am unable to tell if *V. sp.* is conspecific with *V. serrilineata* since the original description of *V. serrilineata* is utterly scarce, and Zhou et al lack important diagnostic meristic and descriptive information, and do not provide institutional repository numbers for the concerned batch of specimens. Kottelat states in the species account of *V. crassicauda* (2000: 75) that examined material from the Yangbiji River as used by Zhou et al is conspecific with Red River *V. striata*, which is followed herein.

#### ***Beaufortia daon* (Mai, 1978)**



Figure 4 *Beaufortia daon*, KIZ2012000011, 32 mm SL, dorsal, lateral, ventral view, scale bar=5 mm

#### **Examined material**

*Beaufortia daon* (Mai, 1978), KIZ 2012000011, 1 ex., 32 mm SL, Mengyejiang River, Red River basin, Puer Prefecture, Yunnan, China, collected by M. Endruweit, 2011-12-30; EPC0633-4, 2 ex., 29.9–31.3 mm SL, Mengyejiang River, Red River basin, Puer Prefecture, Yunnan, China, collected by M. Endruweit & Jing Wang, 2009-12-30.

#### **Diagnosis**

*Beaufortia daon* is distinguished from its congeners by the following set of characters: Snout obtusely rounded; mandibular structure without central concave; anus not covered by pelvic fins; caudal fin obliquely emarginated; body and head dark brown with paler spots; branched pectoral fin rays 25–26; branched pelvic fin rays 19–22; lateral line scales 75–85. The three concerned specimens meet all morphological characters. Branched pectoral fins rays were counted to 25–28 in EPC0633, while the other two specimens are within the specified range; branched pelvic fin rays were counted to 19–21 and are well within the specified range; lateral line scales were counted to 76–80 (Mai, 1978; Kottelat, 2001 [English translation of Mai, 1978]).

#### **Habitat and distribution**

Mai (1978) reported *Beaufortia daon* to be very common in streams of Phong Tho and Nam Na, Lai Chau Province, northern Vietnam. This region is located within the Black River (Lixianjiang River) subbasin and is very close upstream to the Mengyejiang River, where all three concerned specimens were collected. Although many days were spent fishing the Mengyejiang along different stretches just three specimens of *B. daon* were yielded. Local fishermen confirmed the scarcity of this fish. In December 2009 the Mengyejiang was approximately 40 m wide and 3 m deep with plenty of 0.40 m deep riffles over smooth rocks and boulders. There were backwaters with plenty of green algae and few patches of a *Potamogeton* species as the only vascular water plant. The water was clear and ran moderate to fast; its parameters were measured to 17 °C, pH 8.8, and 170 µS/cm on 2009-12-30 at 16:00. Two years later on 2011-12-28 at 09:00 am water parameters were 15 °C, pH 7.9, and 150 µS/cm. Syntopic balitorid fishes were *Vanmanenia tetraloba* (Mai, 1978), *Sinogastromyzon tonkinensis* Pellegrin & Chevey, 1935, and a not yet identified *Balitora* species. The range of natural occurrence of *Beaufortia daon* is hereby extended northwards into Chinese Yunnan Province.

#### **Remarks**

In addition to the description of morphological features Mai (1978) also provides a set of morphometrics.

But this information is of little value, since methods for measuring and counting are not explained and value ranges are not provided. An unambiguous repeatability is thus not assured. Mai gives head length/SL, eye diameter/head length, and interorbital distance/head length; all in percentages. The species lot he examined had 48–65 mm SL, while the specimens I examined were smaller (29.9–32 mm SL). It is unclear if Mai considered the dorsal, lateral or abdominal head length as head length. In considering the dorsal head length span linearly from the tip of snout to the posteriormost tip of occipital, the specimens I examined have the following output; head length/SL 26.2 (range: 25.6–26.8 [vs. 21.4%]), eye diameter/head length 18.8% (zero range [vs. 22.7%]), and interorbital distance/head length 43.8% (range: 42.5–45 [vs. 42.7%]). Although my values differ sufficiently from those provided in the original description, it may not affect the specimens' designation as *Beaufortia daon*. The species account of *Beaufortia daon* starts on page 216 in Mai's original description (1978) and erroneously displays a figure of *Homaloptera (Balitora) brucei*. The figure corresponding to *B. daon* is depicted on page 238. At the end of the book there are 49 pages giving a plate overview of all species treated in this monograph correctly depicting *B. daon* as figure 100.

Kottelat (2001) erroneously placed *B. daon* in the genus *Pseudogastromyzon* Nichols, 1925, a balitorid genus easily recognized by ventral fins not united to form a sucking disk vs. united to form a sucking disk in *Beaufortia* Hora, 1932. This is corrected in his later erratum (Kottelat, 2005).

### *Monotrete turgidus* Kottelat, 2000



Figure 5 *Monotrete turgidus*, KIZ2012004201, 68.7 mm SL, lateral view, reversed, scale bar=5 mm

*Tetraodon leiurus* (nec Bleeker, 1950) Huang et al, 1988: 179 (Mekong, Xishuangbanna)

*Monotrete leiurus* (nec Bleeker, 1950) Chu & Chen et al, 1990: 274 (Mekong, Xishuangbanna)

*Monotrete leiurus* (nec Bleeker, 1950) Su & Li, 2002: 210 (Mekong, Xishuangbanna)

### Examined material

*Monotrete turgidus* Kottelat, 2000, KIZ2012004201-3, 3 ex., 59.3–68.7 mm SL, Mengla market, Nanlahe River subbasin, Mekong basin, Kunming Institute of Zoology (CAS), China Zoological Society

Xishuangbanna, Yunnan, China, collected by M. Endruweit & Jing Wang, 2010-1-3; EPC0833, 1 ex., 58 mm SL, Nanmuwo River, Nanlahe River subbasin, Mekong basin, Xishuangbanna, Yunnan, China, collected by M. Endruweit, 2011-1-2; KIZ 1964000515-7, 3 ex., 43.3–83.7 mm SL, Mengla, Xishuangbanna, Yunnan, China; KIZ 1974001118-27, 10 ex., 53.8–76.5 mm SL, Man Zhuang, Yunnan, China; KIZ 1986003745-50, 6 ex., 51.7–77.2, Mengla, Xishuangbanna, Yunnan, China; KIZ 1996003059-64, 6 ex., 25.6–62.1 mm SL, Mengla, Xishuangbanna, Yunnan, China; KIZ 2012001384-93, 10 ex., 54.3–88.5 mm SL, Mengla market, Xishuangbanna, Yunnan, China.

### Diagnosis

In total, 39 specimens off seven lots were identified to be conspecific with *Monotrete turgidus*. This species is described as back greenish brown to black, with gradual or abrupt transition to a white abdominal region; head and body including back with numerous small black spots, some of them with a paler center (in life: orange to red); and a conspicuous ocellus or large blotch below dorsal fin origin absent (Kottelat, 2000).

### Habitat and distribution

Specimen EPC0833 has been obtained from a 200 m long, 30 m wide and around 2 m deep pool-like extension of the Nanmuwo River, a left bank tributary of the Nanlahe River within the Mekong basin, south of Mengla. The water was clear and sluggish, and thick algae pads were attached to rocks and boulders. The habitat was surrounded by primary rain forest.

*Monotrete turgidus* is originally described from central Laotian Savannakhet Province, close to the border with Vietnam. It seems to be a widespread species since its natural range of occurrence is given with Mekong basin in Laos and Thailand (Kottelat, 2000). Huang et al (1988) display color photographs of *M. turgidus*, misidentified as *T. leiurus*, and give its distributional range with the Mekong basin in Xishuangbanna. Su & Li (2002) also misidentify this species as *Monotrete leiurus*. The distributional range of *M. turgidus* is hereby extended northwards into Xishuangbanna incorporating the Nanlahe River subbasin.

### Remarks

Zhou (Chu & Chen et al, 1990) list *Monotrete leiurus* (Bleeker, 1950) as the sole tetraodontid species in Yunnan, reported from the Nanlahe River, which is congruent with my observations that there is only one puffer species in Xishuangbanna.

The correct name for this taxon (not for the species) should be *Monotrete leiurus* (Bleeker, 1850). Kottelat

(2011) corrected Bleeker's publication date to December 1850. The genus *Monotrete* has been erected by Bibron in the year 1855. In 1856 Troschel erected the genus *Monotretus*, which is a unnecessary latinization of the generic name *Monotrete*. This is followed by Hollard (1877), who provided another latinization with the generic name *Monotreta*. *Monotrete* as the earliest publication has priority over the two derivatives. Bleeker gave its original description in Latin. The English translation is as follows:

"A *Tetraodon* with oblong body depressed anteriorly, wider than high, 4 to 4.5 times in length; head obtusely depressed, 3.5 to 3 times in body length; snout profile straight or slightly convex; eye diameter about 4.5 times in lateral head length, 2 to 3 times in interorbital distance; nasal papillae long and uniquely bifid; head, opercula, dorsum, flanks and belly all armed with spines, snout and tail smooth; lateral line inconspicuous; dorsal and anal fin are about the same height, broad, blunt, rounded; caudal fin convex; upper body brownish red devoid of spots, below yellow; fins reddish or brownish. D 3/11; P 1/21 or 1/22 or 2/20; A 2/9; C 10; vernacular: Ikan Buntak; habitat: Jakarta, in the ocean and estuaries; length of 5 specimens: 60 mm to 98 mm".

Considering the set of characters given in the original description of the estuarine/marine *Monotrete leiurus* as largely spined, with long nasal bifid papillae and unspotted upper body portions, it is obvious that the Nanlahe River species has been misidentified by Zhou. In addition, the provided figure (Chu & Chen et al, 1990: 275, Figure 268) does not correlate to the original description neither. The correct identification for the freshwater puffer species inhabiting branches of the Nanlahe River is *Monotrete turgidus* Kottelat, 2000.

### *Xenentodon canceloides* (Bleeker, 1854)



Figure 6 *Xenentodon canceloides*, KIZ2012004204, 143.2 mm SL, lateral view, scale bar=10 mm

*Tylosurus strongylurus* (nec van Hasselt, 1823) Chu & Chen et al, 1990: 231 (Red River, Hekou)

### Examined material

*Xenentodon canceloides* (Bleeker, 1854), KIZ 2012004204, 1 ex., 143.2 mm SL, Mengyejiang River, Red River basin, Puer Prefecture, Yunnan, China, collected by M. Endruweit & Jing Wang, 2009-12-30; KIZ 1960000867, 1 ex., 242 mm SL, Hekou, Honghe Prefecture, Yunnan, China (specimen excluded from

meristics and morphometrics).

### Diagnosis

*Xenentodon canceloides* is readily distinguished from its congener *X. cancela* Hamilton, 1822 by postorbital length of head 3.5 (2.95 in KIZ 2012004204) times in preorbital length (vs. 2.5 times in *X. cancela*), enlarged teeth in upper jaw 21–32 (32 in KIZ 2012004204; vs. 9–21 in *X. cancela*), dorsal fin origin slightly posterior to anal fin origin (vs. slightly anterior to anal fin origin), length of upper jaw 2.3–2.6 (2.55 in KIZ 2012004204) times in body length (this is SL minus lateral head length [vs. 2.6–3.2]), TL less than 30 cm (vs. TL exceeding 30 cm [Roberts, 1989; Serov et al, 2006]).

### Habitat and distribution

*Xenentodon canceloides* was found syntopic with *Beaufortia daon*. For notes on the biotope please refer to details given for *Beaufortia daon* (Mai, 1978).

Kottelat (2001) lists *X. canceloides* for Laos, and Mai (1992) provides occurrence details for southern Vietnam. The presence of just two extant specimens in two lots in KIZ reflects its scarcity in Yunnanese waters.

### Remarks

The value of postorbital length in preorbital length in KIZ 2012004204 was measured to 2.95 and lies in between the two specified ranges: 3.5 in *Xenentodon canceloides* and 2.5 in *X. cancela* (Serov et al, 2006). By definition, preorbital length is measure from the tip of the upper jaw to the anterior margin of the orbit. The 143 mm long specimen concerned seems to be a juvenile with a not yet fully developed upper jaw. During ontogenetic development of belonid fishes the lower jaw grows in advance of the upper jaw, which catches up somewhat later with the former. The lateral head length, in particular the preorbital length, is relatively shorter in juveniles than in adults (Fahay, 2007). The specimen concerned has a noticeably shorter upper than lower jaw.

Needlefish of the family Belonidae have *Xenentodon* as the only freshwater genus in Asia (Roberts, 1989), *Strongylura* van Hasselt, 1824 is marine. Zhou (Chu & Chen et al, 1990) reported *Tylosurus strongylurus* (van Hasselt, 1823) from the Red River basin in Yunnan, a species placed in the genus *Strongylura* by Parin (1967). The correct naming of this taxon is *Strongylura strongylura* (van Hasselt, 1823). The fish depicted in Chu & Chen et al (1990: 232, Figure 231) is actually a *Xenentodon*, rather than *S. strongylura*. *Strongylura strongylura* is recognized by a dorsal fin distinctively posterior to the origin of the anal fin (vs. slightly posterior in *Xenentodon*), a prominent black spot present at the caudal peduncle (vs. absent), and 100-130 predorsal

scales (vs. more than 200 [Roberts, 1989; Collette, 1999; Serov et al, 2006]).

Kottelat (2001) confirmed that specimen MNHN 1892-50 from the Black River subbasin in Vietnamese Lai Chau Province belongs to the genus *Xenentodon*. Lai Chau Province is located downstream and rather close to the biotope where the concerned specimen of *Xenentodon canciloides* was collected.

Roberts (1989) and Serov et al (2006) report one or more not yet described *Xenentodon* species. These species have large scales (140-160 predorsal scales vs. more than 200 in *X. cancila* and *X. canciloides*).

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## ***Schistura sexnubes*, a new diminutive river loach from the upper Mekong basin, Yunnan Province, China (Teleostei: Cypriniformes: Nemacheilidae)**

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**Abstract:** An ichthyofaunistic survey of Mekong tributaries in Lincang Prefecture, Yunnan Province, China yielded a new species of nemacheilid loach, herein described as *Schistura sexnubes* species nova. The new species is readily distinguishable from its congeners by the following combination of characters: 8+8 branched caudal fin rays, an incomplete lateral line, a dissociated caudal bar, a shallow caudal peduncle depth (7.6%–9.6% SL; respectively caudal peduncle 1.76–1.95 times longer than deep), a diminutive size of less than 50 mm SL, and no sexual dimorphism. A dorsocephalic pattern consisting of a black, forward directed V-shaped formation located between the nares, and a white, ovoid blotch on the upper operculum serves as an autapomorphy.

**Keywords:** *Schistura sexnubes*; New species; Mekong basin; Nemacheilidae; Yunnan

River loaches of the family Nemacheilidae are a typical ichthyofaunistic element of riverine environments in Southeast Asia. They preferably inhabit small and medium sized streams with swift current over rocky substratum. Evolutionarily, they are a very successful cypriniform family proven by their presence in almost every suitable biotope; there is merely a stream nemacheilid loaches do not populate. Up to six different nemacheilid species are populating suitable biotopes within the upper Mekong basin in Yunnan Province, China. Kottelat (1990: 16) reports the same number from Indochinese streams. This high number of related syntopic species reflects the availability of a variety of suitable different niches within the biotope and a high adaptation to these niches. A high niche adaptation grade minimizes evolutionary interspecific competition and maximizes the survival rate of a certain species. Among river loaches, the grade of rheophilia and substratum preference are apparently crucial factors in determining the distributional range (Endruweit, 2011).

With its remarkable hydrology and geology Yunnan province in Southwest China possesses excellent environmental conditions for the evolution of speciose fish assemblages. Although the upper Mekong basin, referred to as Lancangjiang River in China, is

well known for its species richness (e.g. Zheng, Chen & Yang, 2009), to date just four species of the species-rich nemacheilid loach genus *Schistura* McClelland, 1938 sensu Zhu (1989) have been described from its basin. These are in chronological order of original description *Schistura conirostris* (Zhu, 1982), *S. latifasciata* (Zhu & Wang, 1985), *S. kloetzliae* Kottelat, 2000, and *S. bannaensis* Chen et al, 2005. Further *Schistura* species reported from the upper Mekong in China are *S. porthos* Kottelat, 2000, *S. macrocephalus* Kottelat, 2000, *S. breviceps* (Smith, 1945), *S. kengtungensis* (Fowler, 1936), *S. bucculenta* (Smith, 1945), *S. poculi* (Smith, 1945), *S. conirostris* (Zhu, 1982), and *S. pertica* Kottelat, 2000 (Zhu, 1989; Kottelat, 1990, 2001; Chen et al, 2005; Endruweit, 2011). The genus *Schistura* has degraded to a generic catch-all assemblage over time. It contains far more than one hundred valid taxa and is considered polyphylogenetic. It surely will be split up in several genera once the phylogenetic interrelationships of concerned species are better understood.

An ichthyofaunistic survey of Mekong tributaries in eastern Lincang Prefecture yielded five specimens

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belonging to a new species of the genus *Schistura*, which is described herein.

## MATERIALS AND METHODS

Meristics, morphometrics and related terminology follow explanations given in Kottelat (1990). Measurements are taken point to point with a caliper and recorded to nearest 0.1 mm. Specimens were examined using a monocular Fenglin XSP-06. Location coordinates and altitudes were determined using a Global Positioning System (GPS) Garmin handheld device. Type series material was preserved in the field using 95% industrial ethanol and placed in the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences. A part of the comparative material is in the author's collection (EPC).

### *Schistura sexnubes* sp. nov.

*Schistura latifasciata* (nec Zhu & Wang, 1985), Chen et al, 2010: 400 (Nanzhaihe River, Mekong basin, Shuangjiang County, Yunnan, China)

### Type series material

Holotype; KIZ2011000101, 33.4 mm SL; Mengmenghe River, Xiaoheijiang River subbasin, Mekong basin, Shuangjiang County, Lincang Prefecture, Yunnan Province, China; N23°33.045', E99°51.239', 1047 m a.s.l.; collected by Bisset M & Endruweit M, 2010-10-04.

Paratypes; 4 ex., KIZ2011000102-5, 28.8-37.4 mm SL; KIZ2011000103, dissected, male; same collection details as holotype; 1 ex., KIZ2005012401, 47.9 mm SL, gravid female, Nanzhaihe River, Xiaoheijiang River subbasin, Mekong basin, Mangna village, Gagao power station, Mengku Township, Shuangjiang County, Lincang Prefecture, Yunnan Province, China; N23°40.411', E99°52.142', 1152 m a.s.l.; collected by Chen XY, Pan XF & Yu GH, 2005-04-07, specimen excluded from meristics and morphometrics.

### Comparative material

*Schistura cryptofasciata* Chen, Kong & Yang, 2005, holotype, type # KIZ20026453, database # KIZ2002009647, 60.3 mm SL; 3 ex., paratypes, type # KIZ20026454, 57, 64, database # KIZ2002009649, 51, 55, 40.2–93.5 mm SL; Nandinghe River subbasin, Salween basin, Yongde County, Lincang Prefecture, Yunnan Province, China, collected by Kong DP & Cui GH, 2002-06-12.

*Physoschistura shuangjiangensis* (Zhu & Wang, 1985), 7 ex., EPC1665-71, 32.7–55.2 mm SL; Xiaoheijiang River mainchannel, Mekong basin, Gengma County,

Lincang Prefecture, Yunnan Province, China, collected by Bisset M & Endruweit M, 2010-10-05.

*Schistura kloetzliae*, 6 ex., KIZ2010003114-9, 37.1–48.8 mm SL; 10 ex., EPC1154-63, 38.8–50.6 mm SL; Nanxinghe River, Luosuojiang River subbasin, Mekong basin, Mengla County, Xishuangbanna, Yunnan Province, China, collected by Endruweit M, 2010-12-30; 1 ex., EPC0894, 39.1 mm SL; Luosuojiang River subbasin, Mekong basin, Menglun market, Mengla County, Xishuangbanna, Yunnan Province, China, collected by Endruweit M, 2010-12-30.

Descriptive details including morphometrics and meristics of *Schistura geisleri* Kottelat, 1990 and *S. spilota* (Fowler, 1934) were taken from Kottelat (1990); those of *S. diminuta* originate from Ou et al. (2011) and *S. latifasciata* from Zhu (1989).

### Diagnosis

*Schistura sexnubes* species nova is readily identifiable by the following combination of characters: 8+8 branched caudal fin rays, an incomplete lateral line, a dissociated caudal bar, a shallow caudal-peduncle depth (7.6%–9.6% SL; respectively caudal-peduncle 1.76–1.95 times longer than deep), a diminutive size of less than 50 mm SL, and no sexual dimorphism. A dorsocephalic pattern consisting of a black, forward directed V-shaped formation located between the nares, and a white, ovoid blotch on the upper operculum serves as an autapomorphy.

### Description

For selected morphometrics refer to Table 1.

Fin formula: D iv,8.5; P 11-12; V 9; A iii,5.5; C 8+8.

An elongated nemacheilid; body laterally compressed, body width at dorsal fin origin (8.0%–9.6% SL) and at anal fin origin narrow (4.5%–5.7% SL), body depth low (12.6%–14.1% SL). Pectoral fin long (20.1%–21.6% SL), but does not reach the origin of the pelvic fin; pelvic fin long (16%–18.4% SL), slightly surpassing anus, inserted slightly in front of to opposite to dorsal fin, distal margin of dorsal fin linear, caudal fin emarginated with pointed tips and lower lobe slightly longer than upper, visible in stretched fin condition (Figure 2). Axillary lobe well developed, posteriorly free. Caudal-peduncle depth shallow (7.6%–9.6% SL), without crest. Anus located 1-1.5 eye diameter in front of anal-fin origin, pre-anus length comparatively short (69.8%–72.2% SL).

Body irregularly scaled; patches with minute, cycloid scales distributed from the subdorsal area towards the peduncle, predorsal and ventral area devoid of scales. Lateral line incomplete, extension ranges from the end of the dorsal-fin base to the end of anal-fin base. Stomach sac-shaped, large; intestines with one loop; loop does not reach stomach, creating a Z-shape (Figure 3);

Table 1 Comparison of selected morphometrics of *Schistura sexnubes* sp. nov., *S. cryptofasciata*, *Physoschistura shuangjiangensis* and *S. kloetzi*

	<i>Schistura sexnubes</i> sp. nov.					<i>Schistura cryptofasciata</i>					<i>Physoschistura shuangjiangensis</i>					<i>Schistura kloetzi</i>				
	Paratypes; n=4					n=4					n=7					n=17				
	mean	min	max	SD	Holotype	mean	min	max	SD	mean	min	max	SD	mean	min	max	mean	min	max	SD
SL (mm)	33.1	28.8	37.4	4.0	33.4	66.2	40.2	93.5	22.2	66.2	32.7	55.2	7.5	44.3	37.1	50.6	44.3	37.1	50.6	4.1
in % SL																				
Dorsal HL	19.5	19.3	19.8	0.2	20.7	22.2	20.7	23.6	1.2	21.6	20.1	22.9	1.1	19.0	17.2	21.0	19.0	17.2	21.0	0.9
Lateral HL	21.4	20.9	21.9	0.4	23.9	24.8	23.9	25.6	0.9	23.4	21.8	24.4	1.0	20.6	19.6	22.7	20.6	19.6	22.7	0.8
Predorsal length	52.0	51.4	53.5	1.0	50.3	51.1	50.3	53.1	1.3	51.8	51.2	52.7	0.5	52.9	49.6	55.3	52.9	49.6	55.3	1.5
Head depth (at eye)	10.1	9.5	11.0	0.7	10.7	12.1	10.7	14.2	1.5	11.9	11.0	12.3	0.4	10.8	9.7	12.1	10.8	9.7	12.1	0.7
Head depth (at nape)	11.2	10.5	12.2	0.9	12.4	13.7	12.4	16.4	1.8	14.5	13.9	15.2	0.5	12.5	11.1	13.7	12.5	11.1	13.7	0.7
Body depth	13.3	12.6	13.7	0.5	14.4	17.1	14.4	19.7	2.1	18.7	17.9	20.5	0.9	16.4	13.9	21.3	16.4	13.9	21.3	2.1
Depth caudal peduncle	8.5	7.6	9.6	0.8	11.7	13.5	11.7	15.0	1.4	11.7	11.0	13.0	0.7	12.1	10.8	13.9	12.1	10.8	13.9	0.8
Head width (at nares)	7.6	7.2	8.0	0.4	10.2	13.0	10.2	15.9	2.3	10.3	9.5	13.2	1.3	9.1	7.7	10.4	9.1	7.7	10.4	0.7
Body width (dorsal origin)	8.6	8.0	9.3	0.6	12.7	15.0	12.7	17.4	2.0	13.6	12.5	15.9	1.2	13.0	10.7	15.7	13.0	10.7	15.7	1.4
Body width (anal origin)	5.1	4.5	5.6	0.5	7.7	10.3	7.7	12.3	1.9	8.6	7.4	10.4	1.0	8.1	7.0	10.0	8.1	7.0	10.0	0.9
Eye diameter	4.6	3.7	5.2	0.6	3.0	3.8	3.0	5.0	0.8	4.2	3.9	4.5	0.2	3.7	3.2	4.9	3.7	3.2	4.9	0.4
Interorbital width	5.1	4.8	5.9	0.4	7.0	7.6	7.0	8.3	0.7	8.5	8.0	9.0	0.3	6.5	5.7	7.2	6.5	5.7	7.2	0.4
in % dorsal HL																				
Body depth	68.1	64.4	71.2	3.0	61.1	77.1	61.1	87.4	11.4	86.9	78.3	92.2	5.9	86.5	69.2	109.5	86.5	69.2	109.5	11.7
Depth caudal peduncle	43.6	39.3	48.6	3.9	49.5	61.2	49.5	66.7	8.1	54.4	48.8	59.5	4.4	63.8	55.1	75.0	63.8	55.1	75.0	4.3
Length caudal peduncle	80.0	76.8	85.7	4.0	67.4	76.4	67.4	86.1	9.8	68.2	60.0	74.4	4.6	78.7	70.7	88.6	78.7	70.7	88.6	5.9
Head width (at nares)	39.0	37.3	41.1	1.9	43.2	58.7	43.2	70.4	11.5	48.0	41.3	62.9	7.6	47.7	41.0	53.8	47.7	41.0	53.8	4.3
Body width (dorsal origin)	44.3	41.1	47.1	3.0	53.7	67.9	53.7	77.4	10.7	62.9	55.8	70.9	5.1	68.2	56.0	81.5	68.2	56.0	81.5	7.4
Body width (anal origin)	25.9	23.2	28.6	2.4	32.6	46.7	32.6	54.7	10.1	40.0	33.7	46.4	4.4	42.8	36.9	50.7	42.8	36.9	50.7	3.8
Eye diameter	23.4	19.2	27.1	3.3	14.4	17.2	14.4	21.1	2.9	19.4	17.3	21.6	1.5	19.6	17.0	23.1	19.6	17.0	23.1	1.7
Interorbital width	27.3	24.7	30.4	2.3	30.5	34.4	30.5	37.9	3.2	39.5	36.0	43.2	2.6	34.4	28.2	38.1	34.4	28.2	38.1	2.6
Length pelvic fin	87.9	82.1	92.9	5.2	68.2	71.3	68.2	74.2	2.5	85.7	78.2	96.4	5.8	86.6	76.1	103.6	86.6	76.1	103.6	7.6
Length pectoral fin	108.0	102.7	111.9	3.8	78.4	82.3	78.4	87.4	4.0	102.7	93.5	112.6	7.7	105.2	94.9	116.9	105.2	94.9	116.9	6.8
Ratios																				
Dorsal HL/max head width	1.50	1.43	1.69	0.11	1.27	1.27	1.14	1.46	0.15	1.28	1.14	1.43	0.11	1.37	1.28	1.51	1.37	1.28	1.51	0.06
Body depth/body width	1.47	1.42	1.68	0.11	1.14	1.14	1.10	1.18	0.04	1.39	1.26	1.51	0.08	1.27	1.08	1.48	1.27	1.08	1.48	0.12
Head width/body width	1.44	1.40	1.70	0.14	1.18	1.18	1.14	1.27	0.06	1.26	1.10	1.39	0.09	1.08	0.91	1.26	1.08	0.91	1.26	0.10
Interorbital width/eye diameter	1.21	1.18	1.31	0.15	2.06	2.06	1.45	2.32	0.41	2.04	1.82	2.26	0.13	1.76	1.33	2.19	1.76	1.33	2.19	0.20
Length/depth caudal peduncle	1.86	1.84	1.95	0.09	1.25	1.25	1.09	1.38	0.12	1.26	1.03	1.42	0.14	1.24	1.03	1.46	1.24	1.03	1.46	0.10



Figure 1 *Schistura sexnubes* sp. nov., holotype, KIZ2011000101, 33.4 mm SL, lateral, dorsal, ventral view; scale bar=10 mm



Figure 2 *Schistura sexnubes* sp. nov., paratype, KIZ2011000104, 30.6 mm SL, lateral view, freshly dead

air bladder without free posterior chamber.

Head sharply triangular with a pointed snout when viewed dorsally (dorsal head length 1.43–1.69 times max. head width), dorsal profile of head convex when viewed laterally; head width at nares narrow (7.2%–8.0% SL; 36.2%–41.1% dorsal HL). Eyes not visible when viewed ventrally; eye diameter moderate (3.7%–5.2% SL; 19.2%–27.1% dorsal HL); interorbital width narrow (4.8%–5.9% SL; 24.6%–30.4% dorsal HL). Suborbital flap absent. Mouth inferior and moderately arched. Upper lip thick, smooth and continuous; lower lip thick, slightly furrowed and discontinuous, with a broad median concave widely exposing lower jaw axially. Upper jaw with a broad and deep processus dentiformis entirely covered by upper lip; lower jaw without median incision (Figure 4). Two pairs of rostral and one pair of maxillary barbels; all barbels short, inner rostral barbels reaching corner of mouth, outer rostral barbels clearly surpassing corner of mouth, maxillary barbels reaching vertical line through center of eye.

Largest recorded size is 47.9 mm SL (KIZ2005012401, paratype).

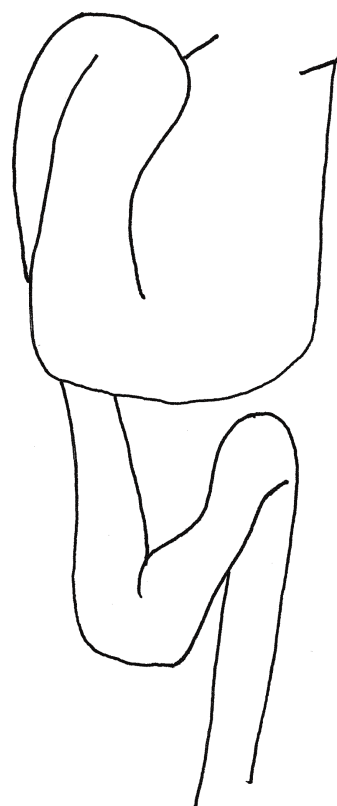


Figure 3 Gastrointestinal tract of *Schistura sexnubes* sp. nov., paratype, KIZ2011000103



Figure 4 Mouth structure of *Schistura sexnubes* sp. nov., paratype, KIZ2011000103

#### Color in ethanol

A distinctive dorsocephalic pattern consisting of a black mask on whitish light gray ground; a black, forward directed V-shaped formation located between the nares; a white, ovoid blotch on the operculum; no pattern in suborbital area despite a thin margin of the cephalic mask (Figure 5). Body with 5–6 irregularly shaped saddle blotches interconnected over dorsal midline and usually somewhat vertically elongated reaching below midline of flank; in some specimens not extending over the ventral midline; usually broadest at dorsal midline.

Some saddle blotches may be dissociated into a saddle and a blotch at flank midline. Saddles in predorsal area sometimes interconnected, generally more irregular than in postdorsal area. Despite 5–6 saddle blotches there is one blotch, located on the lower half of the caudal peduncle as the posteriormost blotch before the basal caudal bar. This blotch stretches from the flank midline and reaches down to the ventral midline, interconnected over the ventral midline in large specimens (KIZ2011000102-3, paratypes). All blotches black on whitish ground coloration. Basal caudal bar dissociated into a distinctive black elongated blotch skewed upwards, upper end showing towards the head, and a smaller faint blackish blotch in the upper half, slightly skewed upwards like the lower blotch. A fine black line along flank midline from head to caudal fin base.

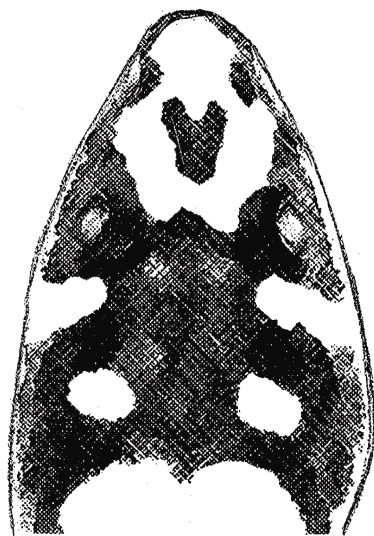


Figure 5 *Schistura sexnubes* sp. nov., dorsocephalic pattern

Dorsal fin with an anterior base spot generated by the third saddle blotch. Dorsal fin with a broad, but faint grayish submarginal band, rest of fin hyaline. Caudal fin with a faint light gray central band. Paired fins and anal fin hyaline with a whitish gloss.

### Life coloration

See Figure 2 for a picture of a freshly dead specimen showing life coloration; like color in ethanol, but ground coloration of flanks and suborbital area silver; ventral area whitish beige; dorsocephalic ground coloration light brown. Dissociated caudal bar pattern with upper blotch not faint, but distinct.

### Etymology

The specific epithet 'sexnubes' is Latin meaning 'six clouds'; an allusion to the color pattern of six saddle blotches, like clouds hanging down the sky; a noun in apposition.

### Ecology

At the time of the visit on 2010-10-04, the Mengmenghe River was 20-30 m wide evincing an elevated water level and highly turbid water typical of the rainy season. There were no submerged plants; the bottom was covered by rocks, pebbles and sand. The biotope seemed to be adversely affected by an overhaul of the neighboring provincial road. *Schistura sexnubes* was found among rocks along the shallow shores in a water depth of 10-40 cm with moderate to fast current. Syntopic benthic species were: Nemacheilidae: *Schistura cryptofasciata*; Sisoridae: *Glyptothorax laosensis*; Clariidae: *Clarias* sp. *Schistura sexnubes* seems to be rare at its type locality. A large batch of nemacheilids consisting of two species was obtained from this location. Around 250 specimens of nemacheilids were collected yielding only five specimens for the type series of *S. sexnubes*. All the rest were identified as *S. cryptofasciata*. The bulk of the predominant *S. cryptofasciata* was given away to locals for food. Hence, the exact number of yielded specimens is unknown.

The Nanzhaihe River, where one of the paratypes was obtained, had a water flow velocity of 0.5 m/s, 21 °C water temperature, and a pH of 5.4 on 2005-04-07 (Chen XY, pers. communication).

The elongated lower caudal fin lobe in *S. sexnubes* indicates niche preference for rapidly running waters over a richly structured bottom substratum, such as rocks and boulders. This specific caudal fin type enables a specimen to rush in high currents from one shelter (crevices between rocks) to another nearby; thereby indicating this species is rheophilous.

### Distribution

*Schistura sexnubes* is only known from the Xiaoheijiang River subbasin, Mekong basin, Shuangjiang County, Lincang Prefecture, Yunnan Province, China (Figure 6).

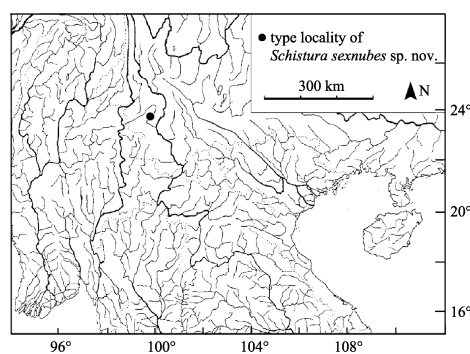


Figure 6 Type locality of *Schistura sexnubes* sp. nov.

## DISCUSSION

The herein described new species *Schistura sexnubes* is readily distinguished by its unique dorso-cephalic color pattern. This autapomorphic characteristic paired with the comparatively small maximum size of less than 50 mm SL makes the species within nemacheilid loaches identifiable at first glance. Further specific characteristics are per diagnosis 8+8 branched caudal fin rays, an incomplete lateral line, a dissociated basal caudal bar and a shallow caudal-peduncle depth (7.6%–9.6% SL; respectively caudal peduncle 1.76–1.95 times longer than deep). Additionally, meristics and morphometrics (see Table 1) support proper identification.

Chen et al (2010) misidentified *Schistura sexnubes* as *S. latifasciata*. According to Zhu (1989) *S. latifasciata* is diagnosed by pectoral fin rays 9–10 (vs. 11–12 in *S. sexnubes*), pelvic fin rays 7–8 (vs. 9 in *S. sexnubes*), caudal fin rays 9+8 (vs. 8+8 in *S. sexnubes*), standard length is 5.3–6.4 times body depth (vs. 7.11–7.96 in *S. sexnubes*), and 3.9–4.4 times head length (vs. 4.84–5.19 in *S. sexnubes*), lateral head length is 5.6–7.6 times eye diameter (vs. 4.06–5.57 in *S. sexnubes*), interorbital width is 1.3–1.9 times eye diameter (vs. 1.00–1.31 in *S. sexnubes*), caudal-peduncle length is 1.1–1.3 times in caudal-peduncle height (vs. 1.76–1.95 in *S. sexnubes*), lateral line complete (vs. incomplete in *S. sexnubes*), and a broad midlateral dark stripe (vs. fine stripe in *S. sexnubes*).

The suborbital flap is an apomorphic character of males of species within the genus *Nemacheilus* Bleeker, 1863 sensu Zhu (1989). There is no species known in which females show this character; hence, it is considered sexual dimorphic. All five type specimens of *Schistura sexnubes* including paratype KIZ2011000103, which was dissected and identified as a male, do not possess a suborbital flap. However, the development of this apomorphic character may be seasonally induced. It may be well developed during the breeding season and in turn reduced after spawning. The breeding season is most probably linked to the beginning of the rainy season in spring providing plentiful food supply for the fry. The type material was obtained in October at the end of the rainy season, at a time when the suborbital flap should be reduced provided that it shows seasonal variation. Just one day after the type material of *S. sexnubes* was obtained, though, the mainchannel of the Xiaoheijiang River yielded *Physoschistura shuangjiangensis* specimens showing a well developed suborbital flap in males. Kottelat (1990: 90) state, that the “absence (of sexual dimorphism) is a character state difficult to use”, which should be respected for identification of loaches in general and applied for the diagnosis of *S. sexnubes* as well. *Nemacheilus shuangjiangensis* was placed in *Physoschistura* by Chen et al (2010).

The large batch size obtained from the Mengmenghe River contained just five specimens of *Schistura sexnubes* herein used as type series material, while *S. cryptofasciata* was abundantly present in all age classes. Both species can be easily differentiated. *Schistura sexnubes* differs from *S. cryptofasciata* in having 8+8 branched caudal fin rays vs. 9+8, an incomplete lateral line vs. complete, and a smaller adult size reaching less than 50 mm SL vs. more than 90 mm SL. Both species also differ notably in morphometrics; lateral head length shorter (20.9%–22.2% SL in *S. sexnubes* vs. 23.9%–25.6% SL in *S. cryptofasciata*), body depth shallower (12.6%–14.1% vs. 14.4%–19.7% SL), caudal-peduncle depth shallower (7.6%–9.6% vs. 11.7%–15.0% SL; 39.3%–48.6% vs. 49.5%–66.7% dorsal HL; respectively caudal peduncle 1.76–1.95 times longer than deep vs. 1.09–1.38 times), head width at nares narrower (7.2%–8.0% vs. 10.2%–15.9% SL; 37.3%–41.1% vs. 43.2%–70.4% dorsal HL), body width narrower (at dorsal origin: 8.0%–9.3% vs. 12.7%–17.4% SL, 41.1%–47.1% vs. 53.7%–77.4% dorsal HL; at anal origin: 4.5%–5.7% vs. 7.7%–12.3% SL, 23.2%–28.6% vs. 32.6%–54.7% dorsal HL), and interorbital width narrower (4.8%–5.9% vs. 7.0%–8.3% SL; 24.6%–30.4% vs. 30.5%–37.9% dorsal HL; respectively interorbital width 1.00–1.31 times eye diameter vs. 1.45–2.32 times). These striking differences rule out that *S. sexnubes* are juvenile *S. cryptofasciata*, as in the case of the former *S. cryptofasciata* was originally described from Lincang Prefecture, but from the adjacent Salween basin. This is the first record from the Mekong basin. Another nemacheilid occurring in both basins in this region is *Schistura poculi* (Smith, 1945).

*Schistura sexnubes* differs from sympatric *Physoschistura shuangjiangensis* in having 8+8 branched caudal fin rays vs. usually 9+8, an incomplete lateral line vs. complete, and in suborbital flap absent vs. present. In terms of morphometrics both species differ in head depth shallower (at eye: 9.5%–11.0% SL in *S. sexnubes* vs. 11.0%–12.3% SL in *P. shuangjiangensis*; at nape: 10.5%–12.6% vs. 13.9%–15.2% SL), body depth shallower (12.6%–14.1% vs. 17.9%–20.5% SL), caudal-peduncle depth shallower (7.6%–9.6% vs. 11.0%–13.0% SL; 39.3%–48.6% vs. 48.8%–59.5% dorsal HL; respectively caudal-peduncle 1.76–1.95 times longer than deep vs. 1.03–1.42 times), head width at nares narrower (7.2%–8.0% vs. 9.5%–13.2% SL; 36.2%–41.1% vs. 41.3%–62.9% dorsal HL), body width narrower (at dorsal origin: 8.0%–9.6% vs. 12.5%–15.9% SL, 41.1%–47.1% vs. 55.8%–70.9% dorsal HL; at anal origin: 4.5%–5.7% vs. 7.4%–10.4% SL, 23.2%–28.6% vs. 33.7%–46.4% dorsal HL), and interorbital width narrower (4.8%–5.9% vs. 8.0%–9.0% SL; 24.6%–30.4% vs. 36.0%–43.2% dorsal HL; respectively interorbital width 1.00–1.31 times eye diameter vs. 1.82–2.26 times).

These considerable differences in meristics and morphometrics rule out the possibility that *S. sexnubes* are juveniles of *P. Shuangjiangensis*.

Phenotypically, *Schistura sexnubes* most closely resembles *S. kloetzliae* Kottelat, 2000, a rheophilous species, which is very common in suitable habitats throughout the Nanlahe River and Luosuojiang River subbasins, Mekong basin, Yunnan Province, China (Endruweit, 2011). The holotype of *S. kloetzliae* (NRM 33199) was obtained from Mengla market, which is within the Nanlahe River subbasin. According to Kottelat (2000) this species also occurs southwards within the Mekong basin in Louangnamtha Province, Laos. Although both species occur in the same basin their distributional ranges do not overlap. *S. sexnubes* and *S. kloetzliae* share 8+8 branched caudal fin rays, and a dissociated basal caudal bar as common characters. In the former species the lateral line is incomplete, but may reach the end of anal fin base, while it is complete or incomplete reaching at least the origin of the anal fin in the latter. Hence, the little overlap in the lateral line extension does not serve to tell these species apart. Morphometrically, *S. sexnubes* can be distinguished from *S. kloetzliae* by body depth deeper (1.42–1.68 vs. 1.08–1.48 times body width), head wider (1.40–1.70 vs. 0.91–1.26 times body width), interorbital width narrower (1.00–1.31 vs. 1.33–2.19 times eye diameter), and caudal-peduncle length longer (1.76–1.95 vs. 1.03–1.46 times caudal-peduncle depth). Furthermore, *S. sexnubes* possesses a basic color pattern of 5–6 irregularly shaped black saddles and a black blotch restricted to the lower half of the caudal-peduncle in front of the basal caudal bar over whitish ground coloration while *S. kloetzliae* have a series of black midlateral blotches, usually interconnected with irregular blotches at dorsal midline and a black bar on the caudal-peduncle in front of the basal caudal bar over beige ground coloration.

*Schistura sexnubes* shares with *S. geisleri* Kottelat, 1990 the dissociated basal caudal bar, the shallow caudal peduncle depth, and the diminutive size of less than 50 mm SL. *S. sexnubes* can be distinguished from *S. geisleri* by having 8+8 vs. 9+8 branched caudal fin rays and the absence of sexual dimorphism vs. presence sexual dimorphism. Males of *S. geisleri* possess a distinctive hammer-shaped suborbital flap and thickened first branched pectoral fin ray. Furthermore, *S. sexnubes* differs from *S. geisleri* in head length shorter (dorsally: 19.3%–20.7% vs. 21.2%–23.5% SL; laterally: 20.9%–22.2% vs. 22.2%–24.9% SL), head depth shallower (at eye: 9.5%–11.0% vs. 11.0%–12.9% SL; at nape: 10.5%–12.6% vs. 12.7%–15.1% SL), body depth shallower (12.6%–14.1% vs. 15.2%–22.0% SL), body width at anal fin origin narrower (4.5%–5.7% vs. 6.3%–10.1% SL), eye diameter smaller (3.7%–5.2% vs. 5.2%–7.1% SL), and interorbital width narrower

(4.8%–5.9% vs. 5.9%–7.5% SL). *S. geisleri* is distributed over upper reaches of the Chao Phraya River basin, Thailand and small drainages in peninsular Thailand. Its range does not overlap with those of *S. sexnubes*.

*Schistura spilota* (Fowler, 1934) is also distributed within the upper reaches of the Chao Phraya River basin and shares with *S. sexnubes* the dissociated caudal bar. *S. sexnubes* is readily distinguishable from *S. spilota* by having 8+8 vs. 9+8 branched caudal fin rays, lateral line incomplete vs. complete, size smaller (less than 40 mm SL vs. reaching at least 88 mm SL), predorsal length longer (51.4%–53.5% vs. 46.8%–50.4% SL), body depth shallower (12.6%–14.1% vs. 15.1%–17.8% SL), caudal-peduncle depth shallower (7.6%–9.6% vs. 10.2%–13.9% SL; respectively caudal-peduncle 1.76–1.95 vs. 1.25–1.53 times longer than deep), head width at nares narrower (7.2%–8.0% vs. 9.4%–13.4% SL), body width at dorsal fin origin narrower (8.0%–9.6% vs. 9.9%–12.9% SL). Neither species possesses sexual dimorphism.

Ou et al (2011) described *Schistura diminuta* from five specimens obtained from the Sekong River, lower Mekong basin, Stung Treng Province, northern Cambodia. With mature individuals featuring a largest recorded size of 19.5 mm SL (paratype, IFReDI, uncat.), *S. diminuta* is considered the smallest species within its genus. The new species *S. sexnubes* possesses a basal caudal bar dissociated into two black blotches, while *S. diminuta* has a pattern reduced to one central, black, ovoid blotch. Apart from the adult size and basal caudal bar pattern there are meristic and morphometric differences; *S. sexnubes* differs from *S. diminuta* in having 8+8 branched caudal fin rays vs. 7+7, body more slender (body width at dorsal fin origin: 8.0%–9.6% vs. 12.8%–14.7% SL; at anal fin origin: 4.5%–5.7% vs. 6.1%–8.3% SL), and interorbital width narrower (4.8%–5.9% vs. 6.6%–7.2% SL). Although both species occur within the same basin, their distributional ranges do not overlap. With a linear distance of more than 1200 km between the two type localities, these species can surely be considered allopatric.

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## Geographic variation in parasitism rates of two sympatric cuckoo hosts in China

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**Abstract:** Rates of brood parasitism vary extensively among host species and populations of a single host species. In this study, we documented and compared parasitism rates of two sympatric hosts, the Oriental Reed Warbler (*Acrocephalus orientalis*) and the Reed Parrotbill (*Paradoxornis heudei*), in three populations in China. We found that the Common Cuckoo (*Cuculus canorus*) is the only parasite using both the Oriental Reed Warbler and Reed Parrotbill as hosts, with a parasitism rate of 22.4%–34.3% and 0%–4.6%, respectively. The multiple parasitism rates were positively correlated with local parasitism rates across three geographic populations of Oriental Reed Warbler, which implies that higher pressure of parasitism lead to higher multiple parasitism rate. Furthermore, only one phenotype of cuckoo eggs was found in the nests of these two host species. Our results lead to two conclusions: (1) The Oriental Reed Warbler should be considered the major host of Common Cuckoo in our study sites; and (2) obligate parasitism on Oriental Reed Warbler by Common Cuckoo is specialized but flexible to some extent, *i.e.*, using Reed Parrotbill as a secondary host. Further studies focusing on egg recognition and rejection behaviour of these two host species should be conducted to test our predictions.

**Keywords:** *Acrocephalus orientalis*; Brood parasitism; Host shift; Egg phenotype before host shift; *Paradoxornis heudei*.

The interaction between the common cuckoo (*Cuculus canorus*) and its hosts is a classic case of coevolution. Cuckoo parasitism exerts intensive costs on the hosts, which evolve anti-parasite behaviour to increase their own reproductive success (Davies, 2000). Parasitism rates of common cuckoo hosts vary extensively among species (Antonov et al, 2006, 2007; Moksnes & Røskaft, 1988; Yang et al, 2010, 2011, 2012) and among different populations within a single host species (Moskát & Honza, 2002; Stokke et al, 2007; Polačiková et al, 2009). Parasitism rate reflects the interaction between cuckoos and their hosts, including the population size of cuckoo and host, host rejection and coevolutionary stage (Davies, 2000).

Here, we investigated the rate of parasitism and multiple parasitisms in two host species of the common cuckoo across three populations in China in an attempt to understand the factors that contribute to differences in parasitism rate. Both the Oriental Reed Warbler

(*Acrocephalus orientalis*) and Reed Parrotbill (*Paradoxornis heudei*) breed in reed habitat and have similar open cup nests. The Oriental Reed Warbler is a summer breeder that was formerly classified as a subspecies of the great reed warbler (*A. arundinaceus*) of western Eurasia (Dyrce & Nagata, 2002). The Reed Parrotbill is a resident species found in eastern China, Mongolia, and Russia (Robson, 2002). Data about the interaction of these two host species with the common

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cuckoo have only rarely been reported before. In this paper we documented and compared parasitism rates of these two species across a large geographic scale among three populations in China.

## MATERIALS AND METHODS

### Study areas

Cuckoo parasitism rates of Oriental Reed Warbler and Reed Parrotbill were quantified in three study sites, namely Chongming Dongtan (CM), Yellow River Delta (YRD) and Zhalong (ZL) National Nature Reserves (Figure 1). CM (N31°25'–38', E121°50'–122°05') is located on Chongming Island, an island at the mouth of the Yangtze Estuary in eastern China. It has a northern sub-tropical monsoon climate with an average annual temperature of 15.3°C. Average annual precipitation is approximately 1,022 mm, with 60% of rainfall occurring between May and September. The wetland types include tidal marshes, tidal flats and shallow open waters, and

birds breeding in CM reed habitats are mainly Oriental Reed Warbler and Reed Parrotbill (Tian *et al.*, 2010). YRD (N37°35'–38°12', E118°33'–119°20') is located on the estuary of the Yellow River in Shandong, eastern China. The mouth of the Yellow River is characterized by huge sediment deposits forming extensive new delta wetland habitats and large areas of reed habitat and birds breeding here are mainly Oriental Reed Warbler and Reed Parrotbill (Li *et al.*, 2011). YRD is characterized by a temperate, semi-humid continental monsoon climate with a mean annual temperature of 12.1 °C. Mean annual rainfall is 551.6 mm, occurring mainly in summer (Cui *et al.*, 2009). ZL (N46°48'–47°31', E123°51'–124°37') lies in the north of Songnen Plain in Heilongjiang, northeast China. This area includes reed swamps, open water and degenerative grasslands. The mean annual temperature and precipitation are 3.2 °C and 426 mm, respectively and birds breeding in ZL include Oriental Reed Warbler, Reed Parrotbill and Black-browed Reed Warbler (*Acrocephalus bistrigiceps*) (Wang *et al.*, 2006).

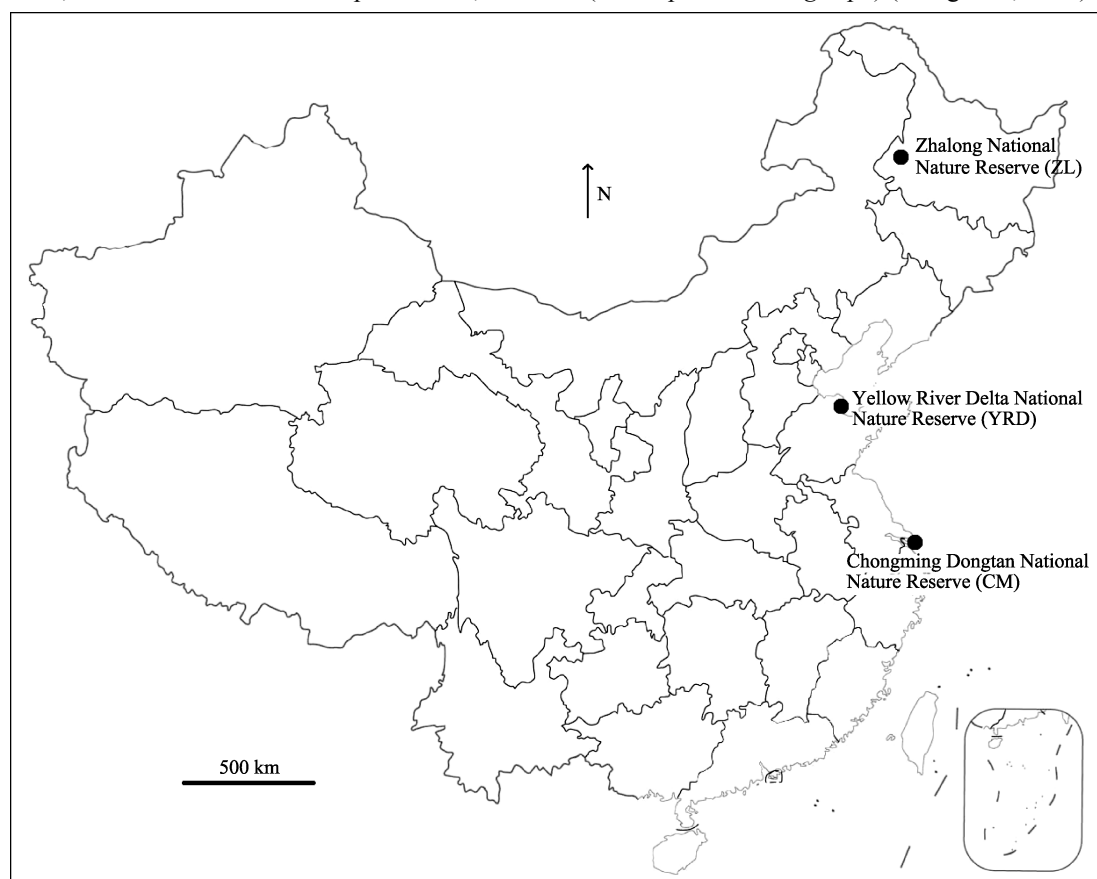


Figure 1 Location of the study sites in China

### Methods

This study was conducted during the breeding season of 2012. We systematically searched for nests of the Oriental Reed Warbler and Reed Parrotbill, which were breeding in sympatric reed habitat at these three

study sites. Nests were checked every day in the egg-laying period and every 2–3 days during incubation. For each nest egg-laying date, clutch size, egg colour, egg size and the occurrence of brood parasitism were recorded. Statistical analysis was done using IBM SPSS Statistics 20.0 (IBM Corp.).

## RESULTS

In total 101, 100, and 107 nests were found in reed habitats of ZL, YRD, and CM, respectively. Nest densities of Oriental Reed Warbler were higher than those of the Reed Parrotbill in all three study sites (Table 1). Only one cuckoo species (the common cuckoo) was recorded in our study sites, and the phenotypes of cuckoo eggs in both Oriental Reed Warbler and Reed Parrotbill nests looked similar (Figure 2), which indicated that parasitism of these two sympatric hosts was caused by the same cuckoo host race. The highest parasitism rate

(34.3%) of Oriental Reed Warbler was recorded in ZL, with 4.1% of multiple parasitism. By contrast, the lowest value was found in CM, with 22.4% of parasitism rate and no cases of multiple parasitism. The multiple parasitism rates were positively correlated with the local parasitism rates across three geographic populations of Oriental Reed Warbler (Spearman's  $\rho$ ;  $r_{sp}=1.00$ ,  $n=3$ ,  $P<0.01$ ). Parasitism rates were much lower in Reed Parrotbill, with 3.6%, 0% and 4.6% in ZL, YRD and CM, respectively. No multiple parasitism was found in Reed Parrotbill nests at the three sites.



Figure 2 Nests of Oriental Reed Warbler (left) and Reed Parrotbill (right) parasitized by common cuckoo eggs (the larger egg in each nest) (Photograph by Guo-Xian LIANG)

## DISCUSSION

The present study showed that three Oriental Reed Warbler populations in China suffered a parasitism rate from 22.4% to 34.3%, which was similar to previous studies in Japan in Oriental Reed Warbler (where it varied from 8% to 39%, see Takasu & Moskát 2011; Moskát et al 2012) and in Europe in Great Reed Warbler *A. arundinaceus* (where it varied from 0% to 31.9%, see Stokke et al 2007; Polačiková et al 2009), with some exception of an extremely high rate (64%) in Hungary (Moskát & Honza 2002). Our results also showed that cuckoo parasitism rates in Reed Parrotbill populations were much lower than that of the Oriental Reed Warbler. Multiple parasitism rates were positively correlated with the local parasitism rates across three geographic populations of Oriental Reed Warbler, which implies that higher pressure of parasitism lead to higher multiple parasitism rate. If we calculated the expected multiple parasitism rates by simply squaring the parasitism rates, the results showed that they were 11.7, 7.4, 5.0 and 0.13, 0.00, 0.20 for Oriental Reed Warbler and Reed Parrotbill, respectively. Obviously the multiple parasitism rates of Oriental Reed Warbler are higher than the observed rates

(Table 1). In contrast, the values for Reed Parrotbill are close to observed rates. This suggests that Oriental Reed Warbler might be better than Reed Parrotbill at defending its nests. Furthermore, only one phenotype of cuckoo eggs was found in the nests of these two host species. Apparently, this cuckoo egg phenotype mimics the eggs of Oriental Reed Warbler. In summary, our results yield two pieces of information: (1) The Oriental Reed Warbler should be the major host of Common Cuckoo in our study areas; and (2) obligate parasitism on Oriental Reed Warbler by Common Cuckoo is specialized but flexible to some extent, i.e. using Reed Parrotbill as a secondary host. We have reported that the Chinese Babax (*Babax lanceolatus*) is exploited as a secondary host by the Large Hawk-cuckoo (*Hierococcyx sparveroides*) (Yang et al 2012). New and naive host populations might be short of egg recognition ability and thus favour the success of cuckoo parasitism. Therefore, it is reasonable to speculate that some individuals of Common Cuckoo shift their host selection to Reed Parrotbill under high pressure of parasitism, or the pressure of egg rejection by Oriental Reed Warbler. Further studies focusing on egg recognition of these two host species should be conducted to test these ideas.

**Table 1 Cuckoo parasitism rates of Oriental Reed Warbler and Reed Parrotbill in three study sites**

Site	Number of host nests	Number of parasitized nests	Parasitism rate (%)	Multiple parasitism rate (%)
Oriental Reed Warbler ( <i>Acrocephalus orientalis</i> )				
ZL	73	25	34.3	4.1
YRD	66	18	27.3	3.0
CM	85	19	22.4	0
Reed Parrotbill ( <i>Paradoxornis heudei</i> )				
ZL	28	1	3.6	0
YRD	34	0	0	0
CM	22	1	4.6	0

In the present study, we did not estimate the densities of these two hosts at these three sites. Previous work showed that host breeding densities or population sizes positively correlated with parasitism rates (Adamík *et al.*, 2009; Stokke *et al.*, 2007). Then nest densities could be one of the key aspects explaining the parasitism rates in our system, and this might be worth considering in future studies.

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Manuscripts should be no longer than 15,000 words, including references and all figures and/or tables. Both British and American English are acceptable, but not a mix of the two. Whenever possible, authors should try to avoid abbreviations or complex technical terminology. When needed, all abbreviations should be standard, abiding by common zoological and genetic nomenclature and using standard unit measurements.

All manuscripts should contain the following information and be organizing in this order:

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The title page should include (i) full title of the paper, not to exceed 250 characters, (ii) full names of all authors with listings of their respective institutional affiliations, (iii) full postal, email, and telephone contact information for the corresponding author; (iv) a brief, running title of no more than 50 characters that will appear at the top of each page.

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Each paper should be preceded by an **abstract** of no more than 250 that presents the purpose, importance, basic methods and key findings of the research. Avoid using abbreviations, citations or references to other parts of the text whenever possible.

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Each paper should include around 5 **keywords** (for indexing), provided immediately below the abstract.

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All tables and figures must be numbered consecutively by Arabic numerals based on the order they appear in the manuscript. The captions to illustrations can be bonded with the tables, and the figure legends should be separated from the illustration. Only high-quality drawings and original photographs (preferably as JPEG or TIFF files) suitable for reproduction can be accepted. Actual enlarging multiples or the length unit (bar, in  $\mu\text{m}$  or nm) should be marked in photos produced by microscopy. Three-line tables (no vertical line) are requested.

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A chromosome is an organized structure of DNA and protein found in cells. It is a single piece of coiled DNA containing many genes, regulatory elements and other nucleotide sequences (Luan et al, 2002). Chromosomes also contain DNA-bound proteins to package the DNA and control its functions (Gwinner, 1990; White, 1977).

Chromosomes vary widely between different organisms (Wang, 2006; Yosida, 1980). The DNA molecule may be circular or linear, and can be composed of 100,000 to over 3,750,000,000 nucleotides in a long chain (Roberts, 1976). Typically, eukaryotic cells (cells with nuclei) have large linear chromosomes and prokaryotic cells (cells without defined nuclei) have smaller circular chromosomes, although there are many exceptions to this rule (Frost, 2011). Also, cells may contain more than one type of chromosome; for example, mitochondria in most eukaryotes and chloroplasts in plants have their own small chromosomes (Kinugawa & Sumihara, 2012).

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Luan YX, Xie RD, Yin WY. 2002. Preliminary study on phylogeny of Diplura. *Zoological Research*, **23**(2): 149-154.

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Roberts PA. 1976. The genetics of chromosome aberration. In: Ashburner M, Novitski E. *The Genetics and Biology of Drosophila*. New York and London: Academic Press, 67-184.

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Frost DR. 2011. *Amphibian Species of the World: an Online Reference*. Version 5.5 (31 January, 2011). New York: American Museum of Natural History, <http://research.amnh.org/vz/herpetology/amphibia/>

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Kinugawa M & Sumihara M. 2012. Negative electrode plate for non-aqueous secondary battery, e.g. lithium ion secondary battery, has sets porosity of surface layer portion of negative mix layer larger than porosity at side of negative electrode collector. Japan, Pat. No. JP2012129040-A. [http://apps.webofknowledge.com/full\\_record.do?page=1&qid=1&log\\_event=no&viewType=fullRecord&SID=R1loI71eBao8GCll5iJ&product=DIIDW](http://apps.webofknowledge.com/full_record.do?page=1&qid=1&log_event=no&viewType=fullRecord&SID=R1loI71eBao8GCll5iJ&product=DIIDW)

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We are pleased to announce that *Zoological Research* has reached a new level this year. On 30 December 2013, the China Academic Journals Electronic Publishing House, Library of Tsinghua University and China Scientific Literature Evaluation and Measuring Research Center jointly awarded *ZR* the “2013 The Highest International Impact Academic Journals of China” in Science, Technology and Engineering for its achievement in internationalizing life sciences research. *ZR* was among 175 Science and Technology Journals selected for the award from a pool of 4622 journals (3502 Science and Technology Journals and 1120 Humanities and Social Science Journals), all of which were in the top 5% of domestic Chinese publications in their field, as determined by comparing their international annual total citation frequency and international impact factor.

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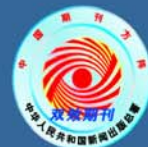
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《动物学研究》编辑部

2014年1月18日

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